SCIENTIFIC OPINION

Scientific Opinion on the request from the USA regarding export of Florida citrus fruit to the EU [1]

EFSA Panel on Plant Health (PLH)[2, 3]

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ABSTRACT

Following a request from the EU Commission, the EFSA PLH Panel conducted a scientific opinion on risk analysis and supporting documents provided by APHIS/USDA in support of the request to remove the Union's plant health import requirement that citrus fruit imported into the EU be sourced from groves where, since the beginning of the last cycle of vegetation no symptoms of citrus canker were observed, neither in their vicinities. The PHL Panel concluded that the transmission of *Xanthomonas citri* subsp. *citri* (*Xcc*) on asymptomatic citrus fruit was more likely when the fruit were collected from infested than from non-infested areas and groves. Symptomatic fruit carries more *Xcc* cells than asymptomatic fruit and the packinghouse disinfectant treatments do not achieve the eradication of *Xcc*. The application of management option 2 (i.e. 'allow distribution of all types and varieties of commercially packed citrus fruit to all US States, subject to packinghouse treatment with APHIS-approved disinfectant. No packinghouse phytosanitary inspection is required') selected by USDA will result in an increase in the *Xcc* load of citrus fruit consignments and in a subsequent increase in the probability of spread of citrus canker through the fruit pathway. Some data provided in the APHIS-USDA documents support that citrus fruit remain a conceptually possible pathway for transmitting and establishing citrus canker disease. The PLH Panel agrees that transmission of *Xcc* from infected fruit to a susceptible host is rare. But the withdrawal of the current EU requirement that citrus fruit imported into the EU be sourced from groves where no symptoms of citrus canker have been observed in the field of production and in its immediate vicinity since the beginning of the last cycle of vegetation, will increase the probability of introduction of *Xcc* into new areas.

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KEY WORDS

Asymptomatic citrus fruit, citrus canker, citrus trade, quarantine, symptomatic citrus fruit, *Xanthomonas citri* subsp. *citri*.

SUMMARY

Following a request from the European Commission, the Panel on Plant Health was asked to deliver a scientific opinion on risk analysis and supporting documents provided by APHIS/USDA in support of the request to remove the Union's plant health import requirement that citrus fruit imported into the EU be sourced from groves where, since the beginning of the last cycle no symptoms of citrus canker were observed, neither in their vicinities.

The Panel developed the opinion in line with the principles described in the document "Guidance of Scientific Committee on transparency in the scientific aspects of risk assessment carried out by EFSA. Part 2: general principles" (EFSA, Scientific Committee, 2009). The principles of Guidance of the Panel on Plant Health following a request from EFSA on evaluation of pest risk assessments and risk management options prepared to justify requests for phytosanitary measures under Council Directive 2000/29/EC have been followed as well (EFSA, Panel on Plant Health, 2009).

The two scientific papers provided by APHIS/USDA supporting their request (Gottwald et al. (2009) and Shiotani et al. (2009)) were analysed and conclusions regarding their scientific aspects were drawn. The Panel conducted the evaluation of the two documents provided by APHIS/USDA (USDA 2009a) and (USDA, 2009b) taking into account the previous EFSA opinion (EFSA, 2006) and referring to the conclusions stated there when relevant.

After having considered all the evidence, the Panel reached to the following conclusions:

- The EFSA PLH Panel recalls that most of the weaknesses of the USDA first document (USDA, 2006) pointed out in its previous opinion (EFSA, 2006) have not been adequately taken into consideration in the subsequent documents produced by USDA-APHIS (USDA 2007a, 2008, 2009a) and therefore remain largely unanswered.

- The new pieces of scientific information, which, according to the USDA fourth document (USDA, 2009a) are provided by the papers from Gottwald et al. (2009) and Shiotani et al. (2009), are not conclusive. Therefore, the EFSA PLH Panel concludes that its previous scientific opinion (EFSA, 2006) is still valid.

With regard to the review of the scientific paper from Shiotani et al. (2009):

The aim of the paper of Shiotani et al. (2009) was to evaluate the phytosanitary risk to importing countries posed by mature Satsuma mandarin fruit harvested from diseased trees by:

- determining the presence of *Xanthomonas citri* subsp. *citri* on these fruit,

- evaluating the potential transmission of the pathogen from fruit to susceptible hosts.

The PLH Panel, after its review concluded that:

- results from Shiotani et al. (2009) studies, where Satsuma mandarin, a citrus species with two resistance characters (i.e. lesser hyperplasia with little rupture of epidermis and lower bacterial population in the tissue) was used, cannot be extrapolated to susceptible citrus cultivars or species,

- in the experiments on the potential of spread of citrus canker from infected Satsuma mandarin fruit within a sweet orange orchard, no information is provided on the susceptibility of the trees during the experiments and little is given on the prevailing environmental conditions

(simultaneous presence of rainfall and susceptible tissues) and the agricultural practices (irrigation, fertilisation *etc*.) applied. The level of *Xanthomonas citri* subsp. *citri* inoculum on the experimental fruit was not monitored at the beginning of the experiments,

- methods and procedures used in this paper missed important information to ensure that the detection of *Xanthomonas citri* subsp. *citri* was truly negative in the experiments. Consequently, it is impossible to draw any consistent conclusions from this paper:, as: (i) the absence of detection by any of the methods used cannot be interpreted due to the lack of a sensitivity level and positive controls associated with the PCR test, (ii) the method used to recover the bacteria from the samples and the selectivity of the culture medium were not appropriate, and (iii) the level of maturity of the sweet orange leaves used in the bioassay was not appropriate to optimize disease expression as they were mature and thus not fully susceptible.

With so many weaknesses in the detection methods and a citrus species that cannot be considered as a relevant model for citrus canker dispersal, the results of this study cannot be transferred to a more general risk assessment of citrus canker.

With regard to the review of the scientific paper from Gottwald et al. (2009):

The paper of Gottwald et al. (2009) is a compilation of various experiments conducted in Florida and Argentina in order to determine:

- the effectiveness of current and modified packinghouse decontamination treatments to reduce the recovery of *Xanthomonas citri* subsp. *citri* from contaminated and infected fruit,

- the epidemiological potential of symptomatic citrus fruit that have passed through the packinghouse undetected to act as a source of inoculum for the infection of susceptible citrus trees in the orchard, and,

- the risk of infection from unprocessed, discarded symptomatic fruit under simulated severe wind-rain conditions.

The PLH Panel, after having critically reviewed the Gottwald et al. (2009) paper, concluded that:

- Occurrence of *Xanthomonas citri* subsp. *citri* on asymptomatic citrus fruit collected in infected orchards is not uncommon, as viable *Xanthomonas citri* subsp. *citri* cells on apparently healthy fruit were detected in some of the experiments.

- The decline observed in the bacterial populations, including those of *Xanthomonas citri* subsp. *citri* after packinghouse treatments was not statistically significant.

- Chlorine applied at the commercial concentration of 200 ppm with or without prewash and/or detergent did not completely disinfect fruit.

- There was a decrease in the *Xanthomonas citri* subsp. *citri* populations in fruit after harvest, but the number of analysed fruit was not large enough, the variability in their bacterial populations was high and the use of numbers of total bacteria as indicators of *Xanthomonas citri* subsp. *citri* survival, was not accurate.

- The experiments on simulated bacterial dispersal from fruit cull piles and fruit suspended in citrus trees suggest that mature citrus fruit are very poor sources of *Xanthomonas citri* subsp.

citri inoculum. Despite the fact that the size/architecture of the canopy and the total leaf area of the trap plants exposed to the wind-driven rain were not comparable with those of mature citrus trees grown in commercial orchards, effective dispersal of *Xanthomonas citri* subsp. *citri* cells did occur, though at a low frequency.

- The experiments on simulated *Xanthomonas citri* subsp. *citri* dispersal were dealing with dispersal by wind-driven rain and not with direct or drip-splash dispersal of *Xanthomonas citri* subsp. *citri* cells from symptomatic fruit discarded on the orchard floor onto the tree canopy. Therefore, the results cannot be extrapolated to a situation where symptomatic fruit/peels have been discarded underneath or in close proximity to susceptible mature citrus trees.

- In many assessments the authors assumed that culturable *Xanthomonas citri* subsp. *citri* cells are the only viable cells ignoring that a viable but non-culturable state (VBNC) of *Xanthomonas citri* subsp. *citri* may also occur. Reliable detection methods (*e.g.* molecular techniques) were not applied to confirm some negative results and to identify *Xanthomonas citri* subsp. *citri*.

- The authors refer most of the time to the results of Shiotani et al. (2009) studies, where the data are not reliable and from which no relevant conclusions can be drawn and ignore the studies of Golmohammadi et al. (2007) which clearly showed that *Xanthomonas citri* subsp. *citri* can survive on packinghouse processed citrus fruit.

With regard to the scientific opinion on the USDA-APHIS 'Updated evaluation of citrus fruit (Citrus spp.) as a pathway for the introduction of citrus canker disease (*Xanthomonas citri* subsp. *citri*)', version May 2009:

The new pieces of scientific information, which, according to the USDA fourth document (USDA, 2009a), are provided by the papers from Gottwald et al. (2009) and Shiotani et al. (2009), are not conclusive (see section 3.1 and 3.2). Therefore, the EFSA PLH Panel concludes that its previous scientific opinion (EFSA, 2006) is still valid.

In the last paragraph of the Executive Summary (USDA, 2009a), the USDA brings the idea that in case typical packinghouse processes are unavailable or when the movement of symptomatic fruit to suitable areas occurs within 24 hours of harvest, the risk of introducing *Xanthomonas citri* subsp. *citri* is reduced only by minimizing the number of symptomatic fruit. This is not supported by any of the information provided by the USDA documents.

After analysing the two provided USDA documents (USDA, 2009a, b), the EFSA PLH Panel concluded that:

- it is likely that, when citrus fruit are permitted for export from areas infested with *Xanthomonas citri* subsp. *citri*, infected fruit do enter into commerce. Moreover, this probability is now even increased in the context of management option 2 retained by the USDA in its rules and regulation.

- significant populations of *Xanthomonas citri* subsp. *citri* can survive packinghouse processes. Moreover, the surviving quantities of inoculum per lot of citrus fruit is now even increased in the context of management option 2 retained by the USDA in its rules and regulation.

- significant populations of *Xanthomonas citri* subsp. *citri* can survive shipment conditions. Moreover, the surviving quantities of inoculum per lot are now even increased in the context of management option 2 retained by the USDA in its rules and regulation.

- fruit with *Xanthomonas citri* subsp. *citri* inoculum may go to areas with climatic conditions suitable for infection. Such conditions are not as rare as described by the USDA (USDA, 2009a). Due to (i) the importation of citrus fruit by all EU Member States, including citrus-producing ones, and (ii) the free circulation of plants and plant products throughout the EU, a significant quantity of citrus fruit imported into the EU may enter citrus-growing areas.

- suitable host plants are present within the EU citrus-producing Member States.

- the risk occurs in the case of asymptomatic citrus fruit originating from infested orchards, and it is even higher in the case of symptomatic fruit.

With regard to the scientific opinion on the USDA-APHIS 'Supplemental risk management analysis of movement of commercially packed citrus fruit from citrus canker disease quarantine area', version May 2009:

The EFSA PLH Panel acknowledges that this document is mainly intended to supplement the previously released RMA document, but its scope is too limited. The EFSA PLH Panel notices that the authors of the USDA sRMA document (USDA, 2009b) disregarded the arguments related to the movement of fresh citrus fruit that had been developed in the previous EFSA opinion (EFSA, 2006) and which remain still valid. In addition, the EFSA PLH Panel recalls that the conclusions drawn by the cited analyses were limited to asymptomatic fruit and thus, they cannot be extrapolated to symptomatic fruit.

The USDA sRMA document (USDA, 2009b) refers to interpretations of the scientific data originating mainly from the Gottwald et al. (2009) and Shiotani et al. (2009) papers. Those two papers, which have already been extensively analysed and evaluated in the first part of this document (see section 3.1. and 3.2.), have shown to be not appropriately documented. In addition to the conclusions withdrawn in sections 3.1. and 3.2., the EFSA PLH Panel concludes that:

- the decline in *Xanthomonas citri* subsp. *citri* population on fruit, reported by Gottwald et al. (2009), was related to the season of sampling rather than the fruit (or lesion) age,

- the efficacy of disinfectant treatments appears quite variable and does not achieve the eradication claimed by the authors.

- none of the references cited by the authors showed that *Xanthomonas citri* subsp. *citri* bacteria do not survive in lesions on harvested fruit long enough to spread the disease to new areas.

- the numerous interceptions of *Xanthomonas citri* subsp. *citri* on citrus fruit originated in infested areas and imported into the EU Member States, and the Golmohammadi et al. (2007) pathogenicity results, are contrary to the authors statement that the storage and shipment conditions reduce the survival of *Xanthomonas citri* subsp. *citri*.

Taking into account its previous opinion (EFSA, 2006), the withdrawal of the USDA systems approach, which was in place until 2007, and the five management options, the EFSA PLH Panel considers that the flexibility to move/export symptomatic and asymptomatic citrus fruit from infested and non-infested orchards, will result in an increase in the *Xanthomonas citri* subsp. *citri* load of citrus fruit consignments and in a subsequent increase in the probability of spread of citrus canker through the fruit pathway.

In addition, the USDA sRMA document (USDA, 2009b) does not propose any method to monitor the efficacy of the selected measures, which is a major failure in the decision scheme.

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BACKGROUND AS PROVIDED BY EUROPEAN COMMISSION

The current European Union plant health regime is established by Council Directive 2000/29/EC on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community (OJ L 169, 10.7.2000, p.1).

The Directive, amongst other provisions, lists *Xanthomonas campestris* (all strains pathogenic to Citrus), hereinafter referred to as citrus canker, amongst harmful organisms of plants, the introduction of which into, and spread within, the Union shall be banned if present on plants, other than seeds, of *Citrus* L., *Fortunella* Swingle, *Poncirus* Raf., and their hybrids. The Directive further stipulates phytosanitary requirements under which fruits of *Citrus* L., *Fortunella* Swingle, *Poncirus* Raf., and their hybrids, originating in third countries can be imported into the Union. One of the requirements, point 16.2 of Annex IV.A.I of Directive 2000/29/EC, lays down the import conditions with regard to citrus canker. Citrus canker is not present in the EU.

On 22 October 2009, the US Department of Agriculture published a rule[4], attached hereunder, allowing, under certain conditions specified therein, a free interstate movement within the US of citrus canker symptomatic citrus fruit originating in areas quarantined because of the presence of citrus canker.

In their letter of 27 May 2010, the US Animal and Plant Health Inspection Service (APHIS) have requested that the EU considers to remove the current requirement that citrus fruit, imported into the EU be sourced from groves where, since the beginning of the last cycle of vegetation, no symptoms of citrus canker have been observed, neither in their vicinities[5]. In support of their request, APHIS refers to the following documents:

"An Updated Evaluation of Citrus Fruit (Citrus spp.) as a Pathway for the Introduction of Citrus Canker Disease (*Xanthomonas citri* subsp.*citri*)" (USDA, APHIS, May 2009)[6],

"Movement of Commercially Packed Citrus Fruit from Citrus Canker Disease Quarantine Area, Supplemental Risk Management Analysis" (USDA, APHIS, May 2009),

T. Gottwald et al. (2009): The epidemiological significance of post-packinghouse survival of *Xanthomonas citri* subsp. *citri* for dissemination of Asiatic citrus canker via infected fruit, Crop Protection 28, 508–524, and

H. Shiotani et al. (2009): Survival and dispersal of *Xanthomonas citri* pv. *citri* from infected Satsuma mandarin fruit, Crop Protection 28, 19–23.

[4] Citrus Canker: Movement of Fruit from Quarantined Areas (Federal Register, 7 CFR Part 301, Vol.74, No.203, p.54431-54445, 22 October 2009). It is referred in this opinion as Federal Register, 2009.

[5] Council Directive 2000/29/EC, Annex IV.A.I., part of the requirements under point 16.2.c

[6] The document is an updated version of a similar study the first version of which was the subject of an earlier question to EFSA (Question N° EFSA-Q-2006-054). It is referred in this opinion as USDA, 2009a.

TERMS OF REFERENCE AS PROVIDED BY EUROPEAN COMMISSION

EFSA is requested, pursuant to Article 29(1) and Article 22(5) of Regulation (EC) No 178/2002, to provide a scientific opinion on the above risk analyses and supporting documents. These were submitted to the Commission by the APHIS/USDA in support of their request to remove the Union's plant health import requirement that citrus fruit imported into the EU be sourced from groves where, since the beginning of the last cycle no symptoms of citrus canker were observed, neither in their vicinities.

In particular, EFSA is requested to determine whether the conclusions of those risk analyses, i.e.:

that asymptomatic fruit (treated or untreated) is not epidemiologically significant as a pathway for introducing citrus canker,

that symptomatic fruit subjected to a specified packinghouse process that includes washing with disinfectants is neither epidemiologically significant as a pathway for introducing citrus canker, and,

that although citrus fruit may remain a conceptually possible pathway for transmitting and establishing citrus canker disease, research shows that extreme, artificial conditions are required to successfully transmit the pathogen from infected fruit to a susceptible host, and that even under these extreme conditions, transmission is rare,

are scientifically justified.

ASSESSMENT

1. Introduction

1.1. Purpose

This document presents an opinion on the scientific papers and documents provided by APHIS/USDA in support of the request to remove the EU plant health import requirement on citrus fruit, prepared by the EFSA Panel on Plant Health, in response to a request from the European Commission.

1.2. Scope

This opinion covers critical reviews of the "Updated Evaluation of Citrus Fruit (*Citrus* spp.) as a Pathway for the Introduction of Citrus Canker Disease (*Xanthomonas citri* subsp. *citri*)"; the "Movement of Commercially Packed Citrus Fruit from Citrus Canker Disease Quarantine Area, Supplemental Risk Management Analysis"; and two supporting documents (Shiotani et al., 2009, Gottwald et al., 2009).

In particular, the determination is made whether the conclusions of those risk analyses are scientifically justified, i.e.:

- that asymptomatic fruit (treated or untreated) is not epidemiologically significant as a pathway for introducing citrus canker,

- that symptomatic fruit subjected to a specified packinghouse process that includes washing with disinfectants is not epidemiologically significant as a pathway for introducing citrus canker, and,

- that although citrus fruit may remain a conceptually possible pathway for transmitting and establishing citrus canker disease, research shows that extreme, artificial conditions are required to successfully transmit the pathogen from infected fruit to a susceptible host, and that even under these extreme conditions, transmission is rare.

1.3. Note on nomenclature

This opinion concerns the Asiatic citrus canker pathogen that will be called later on in this document *Xanthomonas citri* subsp. *citri*. The taxonomy of this bacterium was recently revised (Schaad et al, 2006). The former taxons *X. campestris* pv. *citri* pathotype A or *X. axonopodis* pv. *citri* were elevated to a species rank *X. citri* (Ah-You et al., 2009; Schaad et al., 2006). The name *X. citri* pv. *citri* is sometimes preferred by some authors (Bui Thi Ngoc et al., 2010). When citing some documents, the name of the pathogen will be kept as used by the authors.

2. Data and methodology

2.1. Data and data sources

Literature searches were performed on the ISI Web of Knowledge databases (CAB Abstracts, FSTA, Medline, ISI Web of Science). In addition, Agris and Agricola were also searched. The detailed information about the search strategies and results can be found in Appendix A. The literature searches were performed for publications from 2006 to April 2011 on any aspect related to the citrus fruit

pathway. The literature before 2006 was taken into account in the Opinion of the Scientific Panel on Plant Health on an evaluation of asymptomatic citrus fruit as a pathway for the introduction of citrus canker disease (*Xanthomonas axonopodis* pv. *citri*) made by the US Animal and Plant Health Inspection Service (APHIS) in 2006 (EFSA, 2006) and was also used in this opinion. The abstracts retrieved were then screened and the full paper considered if the study was concerned with infection of citrus fruit by *Xanthomonas citri* subsp. *citri* and the role of fruit as source of inoculum.

Further references and information were obtained from experts, and from citations within the scientific papers found. The sources of all data used for this opinion are listed in References.

2.2. Methodology

The opinion has been developed in line with the principles described in the document "Guidance of Scientific Committee on transparency in the scientific aspects of risk assessment carried out by EFSA. Part 2: general principles." (EFSA, Scientific Committee, 2009). The principles described in this document for risk assessment apply to all the EFSA's scientific outputs. The principles of Guidance of the Panel on Plant Health following a request from EFSA on evaluation of pest risk assessments and risk management options prepared to justify requests for phytosanitary measures under Council Directive 2000/29/EC have been followed as well (EFSA, Panel on Plant Health, 2009).

The two scientific papers provided by APHIS/USDA supporting their request Gottwald et al. (2009): The epidemiological significance of post packinghouse survival of *Xanthomonas citri* subsp. *citri* for dissemination of Asiatic citrus canker via infected fruit, Crop Protection 28, 508–524, and Shiotani et al. (2009): Survival and dispersal of *Xanthomonas citri* pv. *citri* from infected Satsuma mandarin fruit, Crop Protection 28, 19–23) were analysed and conclusions regarding their scientific aspects were drawn. In particular, the described material and methods, experimental design and results and discussion, were analysed, compared with available references and evaluated.

The Panel conducted the evaluation of the two documents provided by APHIS/USDA ["An Updated Evaluation of Citrus Fruit (*Citrus* spp.) as a Pathway for the Introduction of Citrus Canker Disease (*Xanthomonas citri* subsp. *citri*)" (USDA, 2009a) and "Movement of Commercially Packed Citrus Fruit from Citrus Canker Disease Quarantine Area, Supplemental Risk Management Analysis" (USDA, 2009b)] taking into account the previous EFSA opinion (EFSA, 2006) and referring to the conclusions stated there when relevant.

3. Review of the scientific papers and documents provided by APHIS/USDA in support of the request to remove the EU plant health import requirement on citrus fruits

3.1. Review of the scientific paper from Shiotani et al. (2009)

3.1.1. Aims of the paper

The paper of Shiotani et al. (2009) attempts to show that asymptomatic Satsuma mandarin (*Citrus unshiu*) fruit harvested from severely infected trees do not support detectable *Xanthomonas citri* subsp. *citri* cells and that there is no detectable spread of *Xanthomonas citri* subsp. *citri* from contaminated fruit suspended in trees in the rainwater collected beneath the fruit.

3.1.2. Comments on the Materials and Methods

The PLH Panel considers that the technique used in the 2005 studies to extract the *Xanthomonas citri* subsp. *citri* cells from fruit rinds to be used as templates for PCR is not suitable. Bacterial cells do not concentrate in the pellet by centrifugation at only 1,500 g for 10 min; at least 13,000 g must be applied. It is not possible to determine the occurrence of *Xanthomonas citri* subsp. *citri* on fruit using this protocol. As a minimum, testing the rate of recovery of bacteria from suspensions at different concentrations would have been desirable to evaluate the efficiency of this procedure in recovering bacteria.

The PCR procedure used by the authors is that developed by Hartung et al. (1993). This PCR (primer pair 2-3) produced a sensitivity of 10^3 cfu mL^{-1} (Hartung et al. 1993) to 10^2 cfu mL^{-1} from pure cultures (Golmohammadi et al., 2007). To obtain similar sensitivity in citrus fruit, Golmohammadi et al. (2007) indicated that a DNA extraction was required before amplification but no indication of this step is given by Shiotani et al. (2009). The authors present neither a standardization method to evaluate the sensitivity in their conditions (*e.g.* a dilution series with or without fruit tissues) nor the use of a positive control to provide a basis for interpreting the PCR results. In addition, PCR inhibitors are usually released from rinds of citrus fruit and any negative effect on the PCR sensitivity should have been discounted based on preliminary trials. The amount of Taq polymerase utilised in the amplification protocol (0.5 U Ampli Taq per reaction mixture) was half of that used by Golmohammadi et al. (2007) and this could also have played a role in obtaining a lower sensitivity in the PCR reactions. At that time (i.e. 2006), real-time PCR procedures had already been developed to detect *Xanthomonas citri* subsp. c*itri* and were shown to be more sensitive (Golmohammadi et al., 2007; Mavrodieva et al., 2004). Those procedures were not used in this study even though the detection of the pathogen was the key point in answering the objectives.

Pathogenicity testing was done by infiltration into mature attached leaves of Navel oranges. The PLH Panel considers that mature leaves are known to be less susceptible than young leaves (Gottwald and Graham, 1992; Vernière et al., 2003) and therefore, they are less appropriate to detect low levels of bacteria, as would be expected on asymptomatic or symptomatic Satsuma fruit. Similarly, the detection level of this bioassay was not evaluated and it is not possible to interpret a null detection. Gottwald and Graham (1992) showed that an inoculum concentration of 10^4 cfu mL^{-1} in 200 µL was necessary to produce lesions on young susceptible leaves of grapefruit without wounding. In Shiotani et al. (2009) studies, a 30 µL aliquot of inoculum was used for the biossays on mature sweet orange leaves, but the concentration of this inoculum was not provided.

3.1.2.1. Comments on the Experimental design and statistical analysis

Shiotani et al., (2009) examined Satsuma mandarins from severely infected trees to evaluate if *Xanthomonas citri* subsp. *citri* is detectable on the fruit. In 2005 in total 2941 (2208 asymptomatic, 733 symptomatic) fruits were selected and in 2006 further 2011 (1283 asymptomatic, 728 symptomatic). The total severity of the disease was expressed by a severity index which showed that in 2006 the disease was more severe than in 2005 (index 18 instead of 7.5).

Because no information on the sampling scheme was given, it can not be evaluated if the data represent typical disease levels in Japan. The severity index is very artificial and gives little information on the existing severity of the infection. Especially the distribution of the observations across the different disease classes is missing. The average number of lesions was not calculated either.

The total sample size is high, but no stratified information on the severity classes is given. To express the statistical uncertainty of the experiment, the 95% confidence intervals were calculated for the infection rate of Satsuma mandarins with *Xanthomonas citri* subsp. *citri*. The upper limit of the

confidence interval is 0.10% in 2005 and 0.15% in 2006. This means, that no observed detections of *Xanthomonas citri* subsp. *citri* can not exclude an infection up to these limits.

In a second experiment, contaminated and/or infected fruit were put into Navel orange trees as source of inoculum. It was examined if rainwater is a potential means of spreading the bacteria. In this study, the number and selection of examined traps for rainwater is unclear and small (less than 400). The detection limit of sampling beneath the bags with contaminated/infected fruit is unknown. The influence of the amount of rainfall and the dilution effect is unclear. Some detailed information is missing, like time between placement of experimental fruit in the trees/run-off and rainfall or start of rotting. Due to the small sample size, the remaining statistical uncertainty is high. For the various rain events in November 2005 and March 2006, the lack of detection of *Xanthomonas citri* subsp. *citri* in the rainwater traps or of symptoms on the leaves only confirm a possible spread below 1.3% to 3.5% from all bags with contaminated/infected fruit (upper level of 95% confidence interval). The sample size in the further experiments was even smaller resulting in less precise results.

3.1.3. Comments on the Results and Discussion

For the experiments conducted in 2005, the authors reported that the plating technique was not suitable to monitor the *Xanthomonas citri* subsp. *citri* populations in rinds because of the overgrowth of saprophytic bacterial populations on the semi-selective medium. As a consequence, no conclusion can be drawn on these data.

No conclusion can be drawn either on the experiments of 2005 and 2006 on the potential spread of citrus canker disease from Satsuma mandarin fruit. The main concern is related to the use of a rifampicin resistant mutant of *Xanthomonas citri* subsp. *citri* ($KC21Rif_{100}$) that was not previously utilised in Shiotani et al. (2008) and for which no information on citrus fruit colonization, survival or aggressiveness is available. Data on comparative assays using this mutant and a typical wild strain, following their fitness, survival, and virulence on mandarin and orange trees, would be necessary before definitive conclusions could be made. Moreover, the stability of the rifampicin resistance should also have been checked before using the mutant.

The presence of *Xanthomonas citri* subsp. *citri* on artificially infected fruit was not monitored before and during the experiment in the groves. It is possible that mature fruit that had been artificially contaminated by soaking showed a decline in populations of *Xanthomonas citri* subsp. *citri* but from what starting point is not known. In addition, the bacteria on the surface of the fruit did not survive and were only recovered the day after contamination (Table 5 in Shiotani et al., 2009). The PLH Panel notes that in this case also, the procedures used to recover the bacteria (sonication of the rind followed by centrifugation at 1,500 g for 10 min) do not seem appropriate. The lack of survival on the surface of Satsuma mandarin fruit may explain the absence of spread observed from fruit contaminated by soaking (Table 3 in Shiotani et al., 2009).

During the experiment in the orchard at Kuchinotsu, where attached young Satsuma mandarin fruit had been inoculated by pin-pricking with strain $KC21Rif_{100}$ (Rif^R), this strain was recovered three months after inoculation from only three out of 14 lesions – at a recovery concentration of 3×10^3 cfu per lesion or less (Table 4 in Shiotani et al. 2009). The PLH Panel notes that a variation of the phenotype on Satsuma mandarin fruit has been observed depending on the time of inoculation and has led to different types of symptoms and levels of populations in the lesions (Koizumi, 1972). The authors did not describe which type of lesions was present on the fruit at the beginning of the experiment. Early infection type lesions with a ruptured epidermis or late infection type lesions can maintain and produce different numbers of bacteria. This will influence the dispersal potential of the pathogen present on those fruit lesions.

As acknowledged in this paper, and as previously shown (Goto, 1969; Shiotani et al., 2008; Gottwald et al., 1993), Satsuma mandarin (*C. unshiu*) is a citrus species moderately resistant to resistant to citrus canker. As seen from Fig. 1 in Shiotani et al. (2008), symptoms on leaves developing 40 days after prick inoculation were not erumpent, not really canker-like, but more pustule-like. However, lesions can slightly rupture the epidermis of the Satsuma mandarin fruit depending on the period of inoculation (Koizumi, 1972). The rupture of epidermis following hypertrophy and hyperplasia in the parenchyma, is a major event for the release of *Xanthomonas citri* subsp. *citri* bacteria in water (Koizumi, 1976a; 1976b ; 1977). This does not occur efficiently with Satsuma mandarin. Differences in the morphology of citrus canker lesions may account for differences in the amount of inoculum released (Timmer et al., 1991). Lesions with few openings and little hyperplasia may be less conducive to a large release of inoculum. Furthermore, the number of bacteria that could be exuded into water from young canker lesions on grapefruit was about 10^4 to 10^5 cfu mL^{-1} and continued to be exuded at high levels for 24 h (Timmer et al., 1991). Bacteria were found to exude more slowly from older lesions.

In addition, the rate of multiplication of *Xanthomonas citri* subsp. *citri* on *C. unshiu* differed significantly from those on *C. sinensis* (sweet orange, a moderately susceptible to susceptible host) (Shiotani et al., 2008). After 16 days, the bacterial populations decreased on *C. unshiu*, but not on *C. sinensis*. At that time the population per leaf lesion was about 10^8 cfu on sweet orange and about 10^6 cfu on Satsuma mandarin.

As shown in Table 3 (Shiotani et al., 2009), no leaf lesions were observed in experiments performed in November 2005 and March 2006, probably because the weather conditions were not appropriate for disease development, as stated in the paper. This suggests that conclusions should be drawn from only one experiment in 2006. If the results of the experiments shown in Table 2 (Shiotani et al., 2009) were obtained in the same orchards, it is not surprising that bacteria were not collected in rain traps, because the conditions were not favourable for survival and/or because of the use of a mutant with low fitness. As shown in Table 4 (Shiotani et al., 2009), the authors recovered 10^2 - 10^3 cfu/fruit lesion of the Rif^R *Xanthomonas citri* subsp. *citri* population in three out of 14 lesions from six fruits three months after inoculation, which is not a negligible inoculum source.

The authors state that "*X. citri pv. citri cannot survive on rotted fruit (Fulton and Bowman, 1929)*". However, Fulton and Bowman (1929) did not make such a general statement. During their studies, the authors noticed that the development of *Penicillium* spp. on some of the experimental citrus fruit was followed by a decrease in the number of viable canker bacteria recovered from the edge of the rotted area. However, no decrease in the number of viable bacteria was noticed in the firm areas of the fruit.

3.1.4. Conclusions of the review of the scientific paper from Shiotani et al. (2009)

The aim of the paper of Shiotani et al. (2009) was to evaluate the phytosanitary risk to importing countries posed by mature Satsuma mandarin fruit harvested from diseased trees by:

- determining the presence of *Xanthomonas citri* subsp. *citri* on these fruit
- evaluating the potential transmission of the pathogen from fruit to susceptible hosts.

The PLH Panel, after its review concluded that:

- Results from Shiotani et al. (2009) studies, where Satsuma mandarin, a citrus species with two resistance characters (*i.e.* lesser hyperplasia with little rupture of epidermis and lower bacterial population in the tissue) was used, cannot be extrapolated to susceptible citrus cultivars or species.

- In the experiment on the potential of spread of citrus canker from infected Satsuma mandarin fruit within a sweet orange orchard, no information is provided on the susceptibility of the trees during the experiments and little is given on the prevailing environmental conditions (simultaneous presence of rainfall and susceptible tissues) and agricultural practices (irrigation, fertilisation etc.) applied. The level of *Xanthomonas citri* subsp. *citri* inoculum on the experimental fruit was not monitored at the beginning of the experiments.

- Methods and procedures used in this paper missed important information to ensure that the detection of *Xanthomonas citri* subsp. *citri* was truly negative in the experiments. Consequently, it is impossible to draw any consistent conclusions from this paper, as: (i) the absence of detection by any of the methods used cannot be interpreted due to the lack of a sensitivity level and positive controls associated with the PCR test, (ii) the method used to recover the bacteria from the samples and the selectivity of the culture medium were not appropriate, and (iii) the level of maturity of the sweet orange leaves used in the bioassays was not appropriate to optimize disease expression as they were mature and thus not fully susceptible.

With so many weaknesses in the detection methods and a citrus species that cannot be considered as a relevant model for citrus canker dispersal, the results of this study cannot be transferred to a more general risk assessment of citrus canker.

3.2. Review of the scientific paper from Gottwald et al. (2009)

3.2.1. Aims of the paper

The paper of Gottwald et al. (2009) is a compilation of various experiments conducted in Florida and Argentina in order to determine (i) the effectiveness of current and modified packinghouse decontamination measures to reduce the recovery of *Xanthomonas citri* subsp. *citri* from contaminated and infected fruit, (ii) the epidemiological potential for symptomatic citrus fruit that have passed through the packinghouse undetected to act as a source of inoculum for the infection of susceptible citrus trees in the orchard, and (iii) the risk of infection from unprocessed, discarded symptomatic fruit under simulated severe wind-rain conditions.

3.2.2. Comments on the Materials and Methods

3.2.2.1. Prewash experiments

Both of the prewash trials deal only with asymptomatic fruit (grapefruit and lemon), whereas the primary question is not if the bacterium can survive treatment when on the surface of fruit, but when present in lesions or wounds, where chemicals do not have access. As a general point, the efficacy of disinfectant treatments depends on a number of factors including pH, disinfectant concentration, presence of organic matter on the fruit, and frequency of renewal of the disinfectant solution (Dychdala, 1983; Brown and Schubert, 1987). However, even when these factors are optimized, bacteria have still been shown to survive (Stapleton, 1986).

- Experiments conducted in Florida

Experimental grapefruit fruit were collected in January 2007 in Florida. No data were provided on the environmental conditions, treatments in groves, bacterial populations during the time of fruit collection or on the timing of collection in relation to the harvest period. The PLH Panel notes that *Xanthomonas*

citri subsp. *citri* bacteria can fluctuate over the year and decrease during winter. Epiphytic populations of *Xanthomonas citri* subsp. *citri* recovered from symptomatic leaves fluctuated during the day and were generally higher early in the morning in the presence of dew. The recovery from symptomatic leaves also fluctuated throughout the year and populations recovering seemed to decrease in June-July (winter time in Argentina) (Timmer et al., 1996). *Xanthomonas citri* subsp. *citri* natural populations in the lesions observed in Argentina did not strongly fluctuate as the lesions aged until the lesions overwintered and then populations decreased about 100 fold (Stall et al., 1980). This decrease can even be drastic through the winter season in Japan (Koizumi, 1977). Thus a discontinuity appears in *Xanthomonas citri* subsp. *citri* populations in regions where there is a marked winter season. When the winter temperatures are milder, as in a tropical environment, *Xanthomonas citri* subsp. *citri* populations were not strongly affected and decreased approximately by 10 fold (Pruvost et al., 2002).

In Gottwald et al. (2009) studies, collected fruit were treated one day after harvest, but no information was provided on the precise storage conditions of fruit between harvest and application of treatments. The treatment described in the Materials and Methods as "*(4) pre-wash with water plus detergent followed by chlorine immersion*", which, according to the data of Fig. 2A, was the most effective treatment in reducing the number of total bacteria, is mistakenly referred to in the Results as the "*prewash followed by chlorine and detergent*".

- Experiments conducted in Argentina

The date of collecting experimental lemon fruit in Argentina is not given. No explanation is provided on why the control fruit were collected from another orchard. Only three out of the five treatments were similar to those used in the experiment conducted in Florida, *i.e.* (1) untreated control, (2) immersion in chlorine, and (4) pre-wash with water followed by chlorine immersion. Even in these treatments the time during which the fruit were immersed in chlorine was shorter (20 s) than that in the Florida experiment (45 s). No information is provided on the time between harvest and application of treatments or the conditions under which experimental fruit were stored. No information was provided on how the fruit wash solution was prepared (tap water, phosphate buffered saline, etc.). No information is provided on the exact developmental stage of the two leaves used in the bioassays [*e.g.* in the cited reference of Graham and Leite (2004), leaves were injected infiltrated when they reached ¾ full expansion]. The method employed for assessing *Xanthomonas citri* subsp. *citri* population was different (bioassay) compared to that used in the Florida experiment (plating on KCB) and no details are given on the relative sensitivity of each method with respect to *Xanthomonas citri* subsp. *citri*.

3.2.2.2. Packing line experiments

- Experiments conducted in Florida

In the packing line experiments in Florida, two experiments were conducted in 2006 and 2007 with different treatments each. In 2006, fruit were immersed in **chlorine** (200 ppm)[7] for 45 s followed by **detergent (SOPP**) for 30-45 s followed by **water rinse** for 45 s and sprayed with **wax** (shellac-based) **+ imazalil** for 45 s. In 2007, there was a **pre-wash + detergent** for 45 s followed by **chlorine** immersion for 45 s, then **SOPP** spray for 45 s followed by **water rinse** and **wax** (carnuba-based**) + imazalil.**

[7] We assume that the treatments were conducted by using a chlorine solution at 200 µg/L (200 ppm) as reported in the Figure 2 and not at 200 µl/mL as reported in the text and in the legend, in experiments conducted in Argentina and Florida, as well.

The PLH Panel notes that, in the 2006 experiment, chlorine followed by detergent was applied before the water rinse. No explanation is given for following this order of treatments, which is not common in citrus packaging houses (*i.e.* water rinse is followed by chorine immersion).

After processing, fruit were stored at 5-8 ºC and 50% relative humidity (RH). These conditions are different to the commercial citrus storage conditions (4-15 ºC and 90-95% RH) (Ohioline, 2011). Samples were taken from the stored fruit on day 1, 4 and 7 in 2006 and on day 1, 4, 7 and 21 in 2007. The total number of bacteria was estimated following plating on KCB medium (nutrient agar plus kasugamycin 16.0 mg L^{-1}, cephalexin 16.0 mg L^{-1}, and chlorothalonil 12.0 mg L^{-1}) (Graham and Leite, 2004). Bioassays were also performed on two immature leaves of potted grapefruit. Inoculated plants were incubated at 21-27 ºC and 50-60% RH.

- Experiments conducted in Argentina

In the experiments conducted in Argentina, the treatments were: **chlorine** immersion (200 ppm)[8] for 2 min followed by **detergent** for 20 s, **rinsed with water**, coated with a **wax** (shellac and carnuba-based) **+ imazalil** and **dried at 40 ºC** for 1 min and 40 s. Assays were conducted at three harvest times but Figs 5A, B, C and D appear to present pooled data. No analyses are given to support this pooling. Fruit were harvested from two orchards. No information is provided on the conditions under which fruit were stored after harvest and before bioassays were performed.

3.2.2.3. Experiments on survival in fruit wounds

In the experiments on survival in fruit wounds, grapefruits were harvested on 10 April 2006 and inoculated on 3 May 2006, whereas those harvested on 16 April 2007 were inoculated on 7 May 2007. The conditions of fruit storage between harvest and inoculation and post-inoculation are not stated. Inoculation of fruit was performed by needle-pricking using 100 µL of *Xanthomonas citri* subsp. *citri* inoculum. However, no information was provided on the concentration of this inoculum.

3.2.2.4. Experiments on dispersal from discarded fruit

- Simulated dispersal from fruit cull piles and suspended fruit

In the experiments conducted in Florida, incomplete information is provided on disease severity of the experimental fruit (number and diameter of lesions/fruit) and no information is given on the age of citrus canker lesions. The population of viable *Xanthomonas citri* subsp. *citri* bacteria present on fruit lesions at the beginning of the experiment was not determined.

- Dispersal from infected citrus peels

No information is provided on: (i) the age and the total number of lesions present on the four pieces of the grapefruit peel used as an inoculum source, (ii) the environmental conditions prevailing during the experimental period, and (iii) the orchard practices (irrigation, mulching, etc). According to the literature (Peltier, 1920; Koizumi, 1977; Timmer et al., 1991; Pruvost et al., 2002; Bock et al., 2005), the quantity of *Xanthomonas citri* subsp. *citri* cells dispersed in rain splash depends on various factors, such as the age of the lesions and the ambient temperature. Cooler temperatures during winter reduce

[8] We assume that the treatments were conducted by using a chlorine solution at 200 µg/L (200 ppm) as reported in the Figure 2 and not at 200 µl/mL as reported in the text and in the legend, in experiments conducted in Argentina and Florida, as well.

the number of *Xanthomonas citri* subsp. *citri* bacteria in lesions, whereas temperatures between 20 and 30 ºC favour their multiplication (Peltier, 1920). Bock et al. (2005) showed that the number of bacterial cells collected in rain traps at a given time from citrus trees with lesions older than 6 months was lower than that from trees with younger lesions.

3.2.3. Comments on the Results and Discussion

3.2.3.1. General comments

Overall, viable *Xanthomonas citri* subsp. c*itri* cells were detected in apparently healthy fruit in two out of three reported experiments, which indicates that the occurrence of *Xanthomonas citri* subsp. c*itri* on apparently healthy fruit collected in an infected grove is not uncommon. The findings reported by Shiotani et al. (2009) are not tempered by any positive controls indicating a detection threshold, and focus on moderately resistant Satsuma mandarin fruit, which react to *Xanthomonas citri* subsp. *citri* infection differently from susceptible species and consequently they cannot be used to corroborate any data. The experiments conducted to assess the efficacy of packinghouse treatments demonstrated that the bacterial populations, including those of *Xanthomonas citri* subsp. c*itri*, had a tendency to decline. However, no statistically significant reduction was observed after treatments, even when no *Xanthomonas citri* subsp. *citri* were detected. There is no data showing that *Xanthomonas citri* subsp. *citri* is more susceptible to the chemical treatment than the saprophytic microflora. The increase of bacterial population observed after treatment in some samples could be observed for *Xanthomonas citri* subsp. *citri* in other samples as well. There were several observations in the experiments described that corroborate findings on the decreasing *Xanthomonas citri* subsp. *citri* populations in fruit after harvest but the number of analysed fruit was not large enough, the variability in their bacterial populations was high and the use of numbers of total bacteria as indicators of *Xanthomonas citri* subsp. *citri* survival, was not accurate.

However, there are no data that show that *Xanthomonas citri* subsp. c*itri* populations decline to levels that make the infected fruit a non-source of inoculum for infection of susceptible hosts. The experiments on simulated bacterial dispersal from fruit cull piles and suspended fruit suggest that mature citrus fruit are very poor sources of inoculum for infection of susceptible hosts in the orchard. However, in the experiments, the size/architecture of the canopy and the total leaf area of the young grapefruit seedlings (trap plants, 25 cm tall) exposed to the wind-driven raindrops cannot be compared with those of mature citrus trees grown in commercial orchards. The age and size/architecture of the citrus trees affect the quantity of *Xanthomonas citri* subsp. *citri* bacteria sampled in traps during rainfall events (Bock et al., 2005). Therefore, the results of these experiments cannot be extrapolated to a situation where infected symptomatic fruit/peels are discarded on the orchard floor underneath or in close proximity to susceptible mature citrus trees grown in an orchard. However, the experiments showed that effective dispersal did occur, because symptoms developed on one leaf of one plant out of 16 plants and the bacterium was detected in one sample of splash. Dispersal from soil or non-citrus plant material inoculated with saprophytic populations of *Xanthomonas citri* subsp. *citri* has been experimentally reported on susceptible citrus seedlings (Civerolo, 1984; Goto et al., 1978). The minimum level of *Xanthomonas citri* subsp. *citri* for infection was around 10^2 cfu/g of sample of plant debris.

The authors did not use molecular techniques to confirm some negative plating results and in many experiments they assume that culturable *Xanthomonas citri* subsp. *citri* cells are the only viable cells. This cannot be concluded when taking into account the information from Del Campo et al. (2009) about the induction of a viable but non-culturable state (VBNC) in this bacterium by copper and probably by other inducing factors.

3.2.3.2. Specific comments

- Survival of *Xanthomonas citri* subsp. *citri* on fruit before and after packing line processing

The authors indicated that in the lemon prewash trial "*there was a trend suggesting that chlorine treatment slightly reduced the number of lesions recovered*". However, in Fig. 2B, the mean log numbers of lesions are the same, irrespective of the treatment applied. There is no such a trend presented in Fig. 2B, as chlorine applied alone (2nd bar) did not differ from the control. They further added "*chlorine treatment after prewashing the fruit, with or without detergent was beneficial in reducing the number of Xcc recovered from the fruit*". In Fig. 2B, the mean log numbers of lesions seemed to differ, however when the data are expressed in number of lesions, they are about 1.25 lesions for the control and 1.14 lesions for the less efficient treatment. Chlorine following the application of detergent (without any prewashing, see Fig. 2B, 3rd bar) did not differ from the above-mentioned treatments. There is some inconsistency between what is described in the Materials and Methods and in the Results. In general, treatments did not differ significantly from the control. Data presented in Fig. 2B are questionable as: treatments (3) and (5) on the x axis are different compared to those mentioned under the Materials and Methods. More specifically, treatment (3) should be chlorine followed by detergent, and treatment (5) pre-wash with water followed by chlorine, followed by detergent.

- Packing line experiment for grapefruit

In the packing line experiment of 2006, the highest population of *Xanthomonas citri* subsp. *citri* in the sample on day 7 in cold storage could be accounted for by two fruits that had lesions with high viable *Xanthomonas citri* subsp. *citri* populations and produced 1000 lesions in the bioassay. The finding of few fruit, (among the three replicate samples of only five fruits) confirms the frequent existence of fruit with lesions harboring high numbers of *Xanthomonas citri* subsp. *citri*.

It was claimed by the authors that "*the proportion of bioassay inoculation sites that produced bacteria declined with time (Fig. 3C), suggesting that the potential of lesions on fruit overall to generate Xcc declined with time*". However, there was a slight increase and not a decrease with time post-processing. The proportion of infiltration sites on the bioassayed plants that developed citrus canker lesions declined between the pre-treatment sampling (0.53%) and the day 1 post-packing (0.05%), but slightly and continuously increased until day 7 (0.11%). These data suggest that *Xanthomonas citri* subsp. *citri* can survive the treatment and even slightly increase during storage (as also shown when counting the total bacteria population).

In Fig. 3A, the first bar shows that *Xanthomonas citri* subsp. *citri* was present in apparently healthy fruit from healthy trees. In Fig. 3B, although no significant differences were observed in the level of *Xanthomonas citri* subsp. *citri* population between pre-processed and processed fruit, there was an increase in *Xanthomonas citri* subsp. *citri* population with time in cold storage (1 to 7 days). In Fig. 3E, fruit sampled pre-processing had significantly higher bacterial populations compared to fruit after 1 day in storage but the populations did not differ significantly from fruit stored for 4 or 7 days. There was an increase in total bacteria population with time in cold storage (1 to 7 days).

The 2007 treatment was not a replicate of that of 2006 because both treatments differed in their application. There was an additional preliminary step in 2007: washates from the symptomatic fruit from an infected tree produced the highest log-transformed number of lesions (0.19 lesions/leaf). This value (see Fig. 4A) corresponds to about 1.5 lesions/leaf. However, there was a significant effect of days in cold storage on bacterial populations. Fruit sampled prior to processing, and on day 21 in cold storage had significantly higher bacterial populations compared to post-processed fruit sampled on other days in between (Fig. 4E), showing a similar trend to the data from 2006 and most likely due to an increase in residual populations of general surface bacteria (non-*Xanthomonas citri* subsp. *citri*) on the

fruit in cold storage subsequent to the processing. The population of total bacteria on healthy fruit (3.17×10^4 cfu mL^{-1}) was similar to that on the symptomatic fruit (2.43×10^4 cfu mL^{-1}). Nevertheless, the washates from apparently healthy fruit did not produce canker lesions in susceptible grapefruit leaves, whereas washates from symptomatic fruit produced 10 lesions on leaf confirming the results of the 2006 trial. It is surprising that the total number of bacteria is similar and only composed of other bacteria on healthy fruit. Observations of colonies on KCB medium should be indicative of the genus. Infiltration from colonies isolated from KCB would have been useful to identify these bacteria and check whether they were saprophytic or pathogenic xanthomonads.

In the 2006 experiment, the total bacterial population, which included *Xanthomonas citri* subsp. *citri*, had about the same profile (Fig 3D) as the quantity of *Xanthomonas citri* subsp. *citri* measured by infiltration (Fig 3A) for the different treatments. That was not the case in 2007 making it uncertain that the total bacterial population, as measured by the authors, can represent the *Xanthomonas citri* subsp. *citri* population accurately, thus making it difficult to draw conclusions on the efficacy of the disinfection treatments.

- Packing line experiment for lemon

The greatest number of lesions in bioassays was produced from pre-processed fruit and the least from fruit stored for 7 d in cold storage (Fig. 5B), but there was no significant difference between pre-processed fruit and fruit that was 1 and 4 d in cold storage, which indicates that treatments had no effect.

The proportion of bioassayed injection–infiltration sites that produced bacteria showed a pronounced decline with time (Fig. 5C), consistent with the results of the 2006 and 2007 Florida trials. The proportion of infiltration sites was quite similar pre-packing and 4 days post-packing (Fig 5C). The injection–infiltration bioassay showed that processing fruit through a packing line reduced the activity of canker lesions by approximately 50%, as shown by the mean log number of recoverable *Xanthomonas citri* subsp. *citri* bacteria from packing line treated vs. non-packing line treated samples (F1/4 9.62, Pr >F1/4 <0.0021, Fig. 5D). When the data are expressed in number of lesions, there were about 1.48 lesions and 1.1 lesions for the pre-pack and the packinghouse treatments, respectively, and it could be questioned whether this difference is biologically relevant.

The conclusions drawn by the authors were based on the results of the 2006 experiment only, as those of 2007 were very variable.

According to the authors, the results demonstrated that packing line processing *per se* reduced activity of canker lesions by approximately 50%. Samples that had not passed through the packing line had more active lesions, which suggests that if infected fruit passes through a packing line, the ability of the lesions to produce bacteria is significantly reduced. However, fresh citrus fruit with canker-like symptoms imported from different South American countries have been intercepted by the European Union inspectors in the last years (see Appendix B, Table 1) and *Xanthomonas citri* subsp. *citri* was identified even though these fruit received a post-harvest treatment (as indicated by their certificates). According to Golmohammadi et al. (2007), 16 bacterial isolates from 11 different samples were found to contain viable *Xanthomonas citri* subsp. *citri*. This shows that, under routine conditions, the standard packing line treatment may reduce the number of *Xanthomonas citri* subsp. *citri* bacteria, but the procedure is not fully effective.

In the combined conclusions drawn from the Florida and Argentina packing line experiments, the authors assume a reproducible reduction in *Xanthomonas citri* subsp. *citri* viability, which, however, is not demonstrated in the data from Florida. This is because only data from total culturable bacteria are

shown, rather than the *Xanthomonas citri* subsp. *citri* viable bacteria that could provide the necessary information for drawing conclusions.

3.2.3.3. Survival of Xanthomonas citri subsp. citri in fruit wounds

The concentration of inoculum prepared from the 50 canker lesions was evaluated but the value was not given. The values of populations of bacteria are not easily readable in Fig. 7A & B because of a y axis that is not regular. In addition, Fig. 7A & B seem to show data on total bacteria recovered and not on *Xanthomonas citri* subsp. *citri* viable bacteria that could be the inoculum source, although they are indicated as *Xanthomonas citri* subsp. *citri* in the Figure legend. Consequently it is difficult to arrive at a valid conclusion.

3.2.3.4. Dispersal of Xanthomonas citri subsp. citri

- Dispersal from fruit in discarded cull piles

The studies on dispersal are dealing with dispersal of *Xanthomonas citri* subsp. *citri* by wind-driven rain and not with direct or drip splash-dispersal of *Xanthomonas citri* subsp. *citri* bacteria from infected, symptomatic fruit on the orchard floor onto the tree canopy. The authors observed the recovery of *Xanthomonas citri* subsp. *citri* from one splash at a distance of 2 m from the suspended fruit. *Xanthomonas citri* subsp. *citri* was confirmed by a serological positive test using Agdia immunostrips, a less sensitive and less specific test than Indirect Immunofluorescence (IFI). In Tucumán, the IFI test resulted in a false positive. This makes the point that the methods are not standardized and should have been selected to make the results more easily interpretable.

- Natural dispersal from infected citrus peel

In the natural dispersal experiment, about 5×10^5 to 2×10^6 cfu mL^{-1} of non-*Xanthomonas citri* subsp. *citri* bacteria were recovered from canker lesions throughout the 36 days period, but apparently only one fruit per sampling date was analysed. No symptoms developed on grapefruit seedlings after leaf infiltration when peels were placed in the field for more than 1 day. The interpretation of these results is problematic, particularly given the lack of information on the experimental conditions (see comments under Materials and Methods), and because the bacteria could be either dead or viable but non-virulent.

3.2.4. Conclusions of the review of the scientific paper from Gottwald et al. (2009)

The paper of Gottwald et al. (2009) is a compilation of various experiments conducted in Florida and Argentina in order to determine:

- the effectiveness of current and modified packinghouse decontamination treatments to reduce the recovery of *Xanthomonas citri* subsp. *citri* from contaminated and infected fruit,

- the epidemiological potential for symptomatic citrus fruit that have passed through the packinghouse undetected to act as a source of inoculum for the infection of susceptible citrus trees in the orchard, and,

- the risk of infection from unprocessed, discarded symptomatic fruit under simulated severe wind-rain conditions.

The PLH Panel, after having critically reviewed the Gottwald et al. (2009) paper, concluded that:

- Occurrence of *Xanthomonas citri* subsp. *citri* on asymptomatic citrus fruit collected in infested orchards is not uncommon, as viable *Xanthomonas citri* subsp. *citri* cells on apparently healthy fruit were detected in some of the experiments.

- The decline observed in the bacterial populations, including those of *Xanthomonas citri* subsp. *citri* after packinghouse treatments, was not statistically significant.

- Chlorine applied at the commercial concentration of 200 ppm with or without prewash and/or detergent did not completely disinfect fruit.

- There was a decrease in the *Xanthomonas citri* subsp. *citri* populations in fruit after harvest, but the number of analysed fruit was not large enough, the variability in their bacterial populations was high and the use of numbers of total bacteria as indicators of *Xanthomonas citri* subsp. *citri* survival, was not accurate.

- The experiments on simulated bacterial dispersal from fruit cull piles and fruit suspended in citrus trees suggest that mature citrus fruit are very poor sources of *Xanthomonas citri* subsp. *citri* inoculum. Despite the fact that the size/architecture of the canopy and the total leaf area of the trap plants exposed to the wind-driven rain were not comparable with those of mature citrus trees grown in commercial orchards, effective dispersal of *Xanthomonas citri* subsp. *citri* cells did occur, though at a low frequency.

- The experiments on simulated *Xanthomonas citri* subsp. *citri* dispersal were dealing with dispersal by wind-driven rain and not with direct or drip-splash dispersal of *Xanthomonas citri* subsp. *citri* cells from symptomatic fruit discarded on the orchard floor onto the tree canopy. Therefore, the results cannot be extrapolated to a situation where symptomatic fruit/peels have been discarded underneath or in close proximity to susceptible mature citrus trees.

- In many assessments the authors assumed that culturable *Xanthomonas citri* subsp. *citri* cells are the only viable cells ignoring that a viable but non-culturable state (VBNC) of *Xanthomonas citri* subsp. *citri* may also occur. Reliable detection methods (*e.g.* molecular techniques) were not applied to confirm some negative results and to identify *Xanthomonas citri* subsp. *citri*.

- The authors refer most of the time to the results of Shiotani et al. (2009) studies, where the data are not reliable and from which no relevant conclusions can be drawn and ignore the studies of Golmohammadi et al. (2007), which clearly showed that *Xanthomonas citri* subsp. *citri* can survive on packinghouse processed citrus fruit.

4. Analysis of USDA/APHIS documents

The EFSA PLH Panel was provided with a set of three documents prepared by APHIS/USDA:

- an updated evaluation of citrus fruit as a pathway for the introduction of citrus canker disease (USDA, 2009a), issued after the release of two new scientific papers (Gottwald et al., 2009; Shiotani et al., 2009) considered to provide new information on the subject,

- a supplemental risk management analysis (USDA, 2009b), detailing the five envisaged citrus canker management options, and,

- the related part of the Federal Register (USDA/APHIS, 2009), stating which management option is officially selected.

Only the first two documents were to be analysed by EFSA PLH Panel according to the request from the EC.

In its previous opinion on the USDA-APHIS document (USDA, 2006) entitled "Evaluation of asymptomatic citrus fruit (*Citrus* spp.) as a pathway for the introduction of citrus canker disease (*Xanthomonas axonopodis* pv. *citri*)" (EFSA, 2006), only the asymptomatic fruit pathway was to be evaluated by the EFSA PLH Panel.

The two USDA documents (USDA, 2009a; b) that the EFSA PLH Panel is requested to evaluate refer to both asymptomatic and symptomatic fruit as pathways for the introduction of citrus canker into a new area. However, the level of risk that fresh citrus fruit (both asymptomatic and symptomatic) represents for the introduction of citrus canker into new areas depends on the management options selected. The *Xanthomonas citri* subsp. *citri* bacterial load of fruit is correlated with the phytosanitary status of the harvested orchards.

Management option 2 (*i.e.* "*allow distribution of all types and varieties of commercially packed fruit to all US States, subject to packinghouse treatment with APHIS-approved disinfectant. No packinghouse phytosanitary inspection is required"*) selected by USDA (USDA/APHIS, 2009) leads to the free movement throughout the United States of America of citrus fruit (both asymptomatic and symptomatic) originating from citrus canker-infested orchards. This implies a much higher bacterial load on fruit compared to the previous systems approach, which among other risk mitigation measures also included pest-free areas.

4.1. Scientific opinion on the USDA-APHIS document 'An Updated Evaluation of Citrus Fruit (Citrus spp.) as a Pathway for the Introduction of Citrus Canker Disease (Xanthomonas citri subsp. citri)', version dated May 2009

For more clarity, this part of the opinion is organised according to the structure of the given USDA document, except for comments on the Executive summary which are logically postponed to the end. In addition, some background information is added at the beginning of this section.

4.1.1. Background information

In March 2006, USDA-APHIS first issued a document called 'Evaluation of **a**symptomatic citrus fruit (*Citrus* spp.) as a pathway for the introduction of citrus canker disease (*Xanthomonas axonopodis* pv. *citri*)' (USDA, 2006). The document concludes that "*it is highly unlikely that citrus canker could be introduced on asymptomatic, commercially produced citrus fruit that have been treated with disinfected dips and subject to other mitigations*". This document is hereafter referred to as the 'USDA first document'.

In December 2006, EFSA published a scientific opinion on the above APHIS document, which, amongst others, concluded that "*where an initial inoculum (of Xanthomonas axonopodis pv citri) load exists, the transmission of Xac in the scheme proposed by APHIS is more likely than with the current systems approach*" (EFSA, 2006). This document is hereafter referred as the 'previous EFSA opinion'.

In April 2007, USDA-APHIS issued a revised version called 'Evaluation of **a**symptomatic citrus fruit (*Citrus* spp.) as a pathway for the introduction of citrus canker disease (*Xanthomonas axonopodis* pv *citri*) version 2' (USDA, 2007a), hereafter referred to as the 'USDA second document'. The document concludes that "***a**symptomatic, commercially produced citrus fruit, treated with disinfectant dips, and subject to other mitigations, is not epidemiologically significant as a pathway for the introduction of citrus canker*".

In December 2008, USDA-APHIS issued 'An updated evaluation of citrus fruit (*Citrus* spp.) as a pathway for the introduction of citrus canker disease (*Xanthomonas axonopodis* pv. *citri*)' (USDA, 2008)', hereafter referred to as the 'USDA third document'. In this document, USDA claims that new research, summarized in two recent publications, *i.e.* Gottwald et al. (in press at that time, now known as Gottwald et al., 2009) and Shiotani et al. (2009), provided additional evidence that addressed the key uncertainties identified in the 2007 analysis (USDA, 2007a). The USDA third document (USDA, 2008) concludes that:

- asymptomatic fruit (treated or untreated) is not epidemiologically significant as a pathway for introducing citrus canker, and

- symptomatic fruit subjected to a packinghouse process that includes washing with disinfectants is also epidemiologically insignificant as a pathway for introducing citrus canker.

The USDA third document (USDA, 2008) also notes that "*minimizing the presence of lesions (i.e. minimizing symptomatic fruit) also reduces the risks of introducing Xcc via the fruit pathway and may be justified when typical packinghouse processes are unavailable or when the movement of symptomatic fruit to suitable areas (areas where the fruit has the potential to come into direct contact with suitable trees and high wind/rain conditions) within 24 hours of harvest are highly likely to occur*".

In May 2009, USDA-APHIS published a new document (USDA, 2009a) called 'An updated evaluation of citrus fruit (*Citrus* spp.) as a pathway for the introduction of citrus canker disease (*Xanthomonas citri* subsp. *citri*)'. This paper is hereafter referred to as the 'USDA fourth document'. In this document, similarly to the USDA third document (USDA, 2008), it is claimed that new research, summarized in two recent publications (*i.e.* Gottwald et al. (2009) and Shiotani et al. (2009)), provided additional evidence that addressed the key uncertainties identified in the 2007 analysis (*i.e.* USDA second document). The USDA fourth document concludes that:

- asymptomatic fruit (treated or untreated) is not epidemiologically significant as a pathway for introducing citrus canker, and

- symptomatic fruit subjected to a packinghouse process that includes washing with disinfectants is also not epidemiologically significant as a pathway for introducing citrus canker.

The USDA fourth document (USDA, 2009a), similarly to the USDA third document (USDA, 2008), also notes that "*minimizing the presence of lesions (i.e. minimizing symptomatic fruit) also reduces the risks of introducing Xcc via the fruit pathway and may be justified when typical packinghouse processes are unavailable or when the movement of symptomatic fruit to suitable areas (areas where the fruit has the potential to come into direct contact with suitable trees and high wind/rain conditions) within 24 hours of harvest are highly likely to occur*" (USDA, 2009a).

The last two USDA documents and particularly the fourth document (USDA, 2009a), which the EFSA PLH is requested to evaluate, clearly enlarge the scope of the evaluation as they deal with all fruit, whether they originate from pest-free or infested orchards, whether they show symptoms or not, and whether they are treated or not in the packing stations.

The USDA fourth document (USDA, 2009a), similarly to the previous three USDA documents (USDA 2006, 2007a, 2008), identified the following five key events as necessary for *Xanthomonas citri* subsp. *citri* to be introduced into a new area on commercial citrus fruit:

Event 1: infected or contaminated fruit are harvested,

Event 2: inoculum associated with fruit survives the packing process,

Event 3: inoculum associated with fruit survives shipment,

Event 4: fruit with inoculum goes to an area with conditions suitable for infection, and

Event 5: inoculum encounters a suitable host and conditions for disease development.

The PLH Panel continues to consider that those events are of core importance for the evaluation of both asymptomatic and symptomatic fruit as pathways for the introduction of citrus canker into a new area. Therefore, for the purpose of this opinion, the PLH Panel considers it appropriate to make its comments following the structure of the USDA-APHIS document. But, as the USDA fourth document is said to only focus on new information not at that time available in the USDA second document (USDA, 2007a, which did not take into consideration points made in the previous EFSA opinion), the EFSA PLH Panel also includes comments related to the USDA second and third documents (USDA, 2007a, 2008) when appropriate.

4.1.2. Introduction of the USDA fourth document (USDA, 2009a)

In the first paragraph, the authors recall the conclusions of the USDA second document (USDA, 2007a), according to which, "*asymptomatic fruit is not epidemiologically significant as a pathway for introducing citrus canker when produced under the conditions of a systems approach*". For drawing this conclusion, the USDA second document (USDA, 2007a) does not seem to have taken into consideration the previous EFSA opinion and its conclusions (EFSA, 2006). Moreover, the USDA second document (USDA, 2007a) has not taken into account the fact that a systems approach is deeply challenged when following the management option 2.

The authors then state that "*the original risk assessment (i.e. USDA, 2006) did not focus on the risks associated with the movement of symptomatic fruit, but the scientific literature analysed in the previous document (i.e. USDA, 2007a) is applicable to characterising the risks associated with the movement of symptomatic fruit*', but provide no scientific evidence to support this statement. Jumping from an opinion on asymptomatic fruit to another one on symptomatic fruit is at least highly offhand.

In that part of the document (USDA, 2009a), the authors report that the document is a supplement to the previous risk assessment (USDA, 2007a), which concluded that "*asymptomatic fruit is not epidemiologically significant as a pathway for introducing citrus canker when produced under the conditions of a systems approach"*. According to the authors, since 2007, a series of new research experiments, summarised in two recent publications (*i.e.* Gottwald et al., 2009; Shiotani et al., 2009), have addressed uncertainties identified in the previous risk assessment (USDA, 2007a). Therefore, the new research justifies the re-evaluation by USDA-APHIS of the previous risk assessment conclusions. The authors also state that the new supplemental document (*i.e.* USDA 2009a) (i) is based on the scientific information presented in the previous risk assessment (USDA, 2007a) and thus, it will primarily focus on new information, and (ii) focuses for the first time on the epidemiological significance of symptomatic fruit as a viable pathway for the introduction of *Xanthomonas citri* subsp. *citri* into a new area.

The Panel notes that the authors of the USDA second document (USDA, 2007a) have not taken into consideration the previous EFSA opinion (EFSA, 2006) in making the above-mentioned conclusions.

4.1.3. Event 1: infected or contaminated fruit are harvested

In this and in the following sections, the conclusions of the USDA second document (USDA, 2007a) are given in grey boxes.

Conclusions of the USDA second document (USDA, 2007a) regarding event 1.
- Xac is present in groves with active infections, or likely to be present in nearby groves from which the bacteria may be introduced by wind-driven rain.
- Infected fruits are likely to be culled due to the presence of lesions or injuries.
- The epiphytic presence of Xac on fruit does not have a significant role in pathogen spread. Xac in symptomless, mature fruit produced using commercial practices is likely to be epiphytic and labile.

The authors of the USDA fourth document (USDA, 2009a) assert that "*there is no new information that expands upon or alter (their) conclusions (as laid down in the second USDA document) regarding the first event".*

The PLH Panel examined the primary evidence that the authors of the USDA risk assessment cited to support the above conclusions and found that the USDA second document (USDA, 2007a) did not provide scientific evidence additional to that included in the USDA first document (USDA, 2006) with the exception of two references: Ploper et al. (2004) and Belasque and Rodrigues-Neto (2000). However, the paper of Ploper *et al.* (2004), which has been cited in the second USDA document (USDA, 2007a) to support the statement that "*commercial operations can be highly effective in removing diseased, damaged, disfigured, and blemished fruit through a combination of culling in both the field and packinghouse*" is an unpublished technical report presented in an IPPC (International Plant Protection Convention) Working Group Meeting held in Argentina in 2004. Ploper et al. (2004) stated that the study concerned fruit whose destination was other than the EU in order to be able to admit a level of disease in the packinghouse different to zero. The authors concluded based on the results of their study that a packing plant with the characteristics of the one evaluated in their study has the capacity to efficiently discard the total quantity of fruit with citrus canker symptoms when processing batches with values close to 4% of affected fruit.

Further analysis (see Appendix C) showed that the given data can only confirm that the daily average of symptomatic citrus fruit rate passing the inspection line is below 0.0042%. The data show a dependence between prevalence (between 0.2% and 4%) and the remaining symptomatic citrus fruit rate (upper confidence interval (CI) between 0.0008% and 0.0042%). But also for a given prevalence below 1% it can not be excluded that the remaining symptomatic citrus fruit rate is up to 0.002%. For the given 8 days in a packing line in Tucumán it can be estimated that 3 to 7 symptomatic fruits may have passed the inspection. Further uncertainties exist to the detection limit of the applied visual inspection, the quantifications made in the study and the application of the same detection method (visual inspection) to evaluate the whole inspection process; a more precise standard would be more appropriate to evaluate the inspection. However, current interceptions in the EU of citrus fruit originated from Argentina and other citrus-producing Third Countries (Golmohammadi et al., 2007; see also Appendix B, Table 1) show that not all the packinghouses in Argentina have the characteristics of the one evaluated in the Ploper et al. (2004) study.

Ploper et al. (2004) made also some calculations to determine an acceptable number of symptomatic fruit per tree to guarantee prevalence below 1%. This procedure is only valid for trees with large amount of fruit (average of 7.5 or more trays per tree) and includes a visual inspection of more than 300 fruits per tree. It is questionable if an inspector will be able to control more than 300 fruits per tree.

The reference of Belasque and Rodrigues-Neto (2000), which has been cited to support the USDA statement that "*Researchers in Brazil sprayed asymptomatic fruit with a bacterial suspension of* 10^6

cfu/ml, resulting in non-recovery of inoculated bacteria after 5 days at room temperature under lab conditions" is an abstract of a paper presented at a Congress. In that abstract the authors assumed that the decrease observed within the first 24 hours in the *Xanthomonas citri* subsp. *citri* population carried as contaminants on citrus fruit, was due to the desiccation resulting from the experimental conditions (*i.e.* fruit were kept in the lab, at room temperature). However, the authors of the paper further noted that "*Xac is an organism known to take an epiphytic form in leaves and citrus tissues, which allows its survival for several months*".

Although it is true that symptoms most often do not develop on mature, unwounded fruit (USDA, 2007a, point 1.2, second bullet on page 13), it does not mean that the bacterium is absent from such mature fruit and that mature fruit cannot play a role in disease spread.

In the USDA third document (USDA, 2008), based on the results of Gottwald et al. (2009) published later, it is asserted that "*the viability of bacteria on fruit or associated with fruit lesions drops rapidly (…) and disappears completely 22 days after harvest (…*)". This assertion is not valid as shown in the part of the present opinion dealing with the evaluation of Gottwald et al. (2009) paper (Section 3.2).

In the USDA second document, the authors assume that "*commercially produced citrus is cultivated under specific pest management practices (… which) include field treatment with copper-based pesticides (…). Grove sanitation is another practice used to reduce disease prevalence. (…) fruit culling procedures will remove symptomatic, injured, or blemish fruit from commercial shipments (…)"* These management practices ('systems approach') are not included in the management option 2 retained by the USDA in its rules and regulations (USDA/APHIS, 2009). Therefore, the EFSA PLH Panel considers that the probability under management option 2 of having much more infected or contaminated fruit in orchards is higher than in the previous systems approach as mitigation measures are not guaranteed, and subsequently the probability of harvesting infected or contaminated fruit is increased.

In the USDA second document (USDA, 2007a), it is claimed, based on experiments conducted in Argentina, that "*extremely low (near zero) number of symptomatic, injured or blemish fruit (reach) the packing bench"*. This is contrary to the current interceptions of citrus fruit originating from Argentina and other citrus-producing Third countries (see Appendix B, Table 1), and information given in Golmohammadi et al. (2007).

Amongst others, and in addition to the fact that either scientific data were not always provided to support assumptions or assumptions were incorrectly related to the papers cited in the USDA second document, the following weaknesses were pointed out by the PLH Panel in its previous opinion (EFSA, 2006):

- no evidence supports the claim that commercial handling of fruit eliminates diseased fruit,

- the efficacy of copper sprays is not demonstrated to be as high as claimed,

- stating that symptoms do not develop on mature fruit does not mean that such fruit are free from the bacterium,

- the meaning of 'significant' or 'insignificant' is unclear in the analysed USDA documents.

These weaknesses are still not properly addressed in the USDA second document and therefore remain valid.

The EFSA PLH Panel continues to consider that it is likely that, when citrus fruit is permitted for export from areas infested with *Xanthomonas citri* subsp. *citri*, infected fruit do enter into commerce. Moreover, this probability is now increased in the context of management option 2 retained by the USDA in its rules and regulations (USDA/APHIS, 2009).

4.1.4. Event 2: inoculum associated with fruit survives the packing process

Conclusions of the USDA second document (USDA, 2007a) regarding event 2.
- Symptomatic fruit are highly unlikely to pass through the packing process.
- Standard packinghouse procedures and post-harvest treatments prescribed by the systems approach will remove and/or devitalize epiphytic populations of the pathogen.

The USDA fourth document recalls the two conclusions already stated in the USDA first (USDA, 2006) and second (USDA, 2007a) documents with respect to Event 2:

1. Symptomatic fruit are highly unlikely to pass through the packing process, and,

2. Standard packing house procedures and post-harvest treatments prescribed by the systems approach will remove and/or revitalize epiphytic populations of the pathogen to the extent that they become epidemiologically insignificant.

The PLH Panel notes that with respect to the second conclusion, the USDA first and second documents (USDA, 2006, 2007a) did not actually include the last part, *i.e.* "*to the extent that they become epidemiologically insignificant*", which means that the USDA third and fourth documents go far further concluding that "*post-harvest treatments (…) remove and/or devitalize epiphytic populations of the pathogen to the extent that they become epidemiologically insignificant*". Even though this conclusion refers only to epiphytic populations, there is nothing either in the USDA second document (USDA, 2007a) or in the USDA third (USDA, 2008) and fourth (USDA, 2009a) documents that supports it.

Notwithstanding the paper from Gottwald et al. (2009), previously discussed, the concerns given in the previous EFSA opinion regarding Event 2 remain mostly unanswered.

The EFSA PLH Panel acknowledges that post-harvest treatments remove or kill part of the populations of *Xanthomonas citri* subsp. *citri*, but points out the partial efficacy of such treatments on the total bacterial populations and their lack of efficacy on symptomatic fruit. Although the authors of the USDA fourth document (USDA, 2009a) recognise that "*both protocols (described by Gottwald et al., 2009) were inconclusive in terms of the ability of the lesions to produce viable bacteria"*, they still consider that it *"do(es) not detract from the previous conclusions (USDA, 2007a) that standard packinghouse procedures and post-harvest treatments (…) will remove and/or devitalize epiphytic populations of the pathogen*". The experiments reported in Gottwald et al. (2009) paper show that the prewash treatment has no significant statistical effect on populations of *Xanthomonas citri* subsp. *citri*.

The authors of the USDA fourth document (USDA, 2009a) also continue not to consider the increased likelihood of a significantly higher amount of *Xanthomonas citri* subsp. *citri* on asymptomatic and symptomatic fruit lots harvested from infested areas compared to those harvested in orchards free of *Xanthomonas citri* subsp. *citri*.

In the USDA second document (USDA, 2007a), it is claimed based on experiments conducted in Argentina that "*zero symptomatic fruit (are) packed in boxes*". This is contrary to the current interceptions in the EU of citrus fruit originated from Argentina (see Appendix B, Table 1) (even though those fruit are originating from sound *Xanthomonas citri* subsp. *citri*-free orchards and are subjected to

strict culling, cleaning and disinfecting) and analyses made by Golmohammadi et al.(2007) on fruit sampled in Spain from consignments imported from Argentina.

Amongst others, the following weaknesses were pointed out in the previous EFSA opinion (EFSA, 2006):

- the likelihood of a significant higher amount of *Xanthomonas citri* subsp. *citri* on fruit collected from infested areas than those originating from orchards free of *Xanthomonas citri* subsp. *citri* is not taken into consideration,

- the efficiency of the fruit process to eliminate *Xanthomonas citri* subsp. *citri* is far from as good as claimed by the USDA,

- USDA ignores the fact that fruit with no visible symptoms may nevertheless carry *Xanthomonas citri* subsp. *citri* as contaminants,

- the protective role of bacterial exo-polysaccharides is not investigated, and

- chlorine treatment is not 100% efficient.

These weaknesses are still not properly addressed in the USDA fourth document (USDA, 2009a) and therefore remain valid.

The EFSA PLH Panel considers that significant populations of *Xanthomonas citri* subsp. *citri* may survive the packinghouse processes. Moreover, the surviving quantities of inoculum per citrus fruit consignment are now even increased in the context of management option 2 retained by the USDA in its rules and regulations.

4.1.5. Event 3: inoculum associated with fruit survives shipment

Conclusions of the USDA second document (USDA, 2007a) regarding event 3.
- Bacteria that survive the packing process will have a high rate of mortality during shipping.
- Bacteria that survive on the fruit's surface or in lesions/injuries associated with fruit, after post-harvest treatment, will not multiply or cause disease development in treated fruit.

Notwithstanding the papers from Gottwald et al. (2009) and Shiotani et al. (2009) previously discussed, the concerns given in the previous EFSA opinion (EFSA, 2006) regarding Event 3 remain mostly unanswered.

The document from Belasque and Rodrigues-Neto (2000) is an abstract, which refers to the viability of *Xanthomonas citri* subsp. *citri* bacteria present as contaminants on the surface of spray-inoculated 'Valencia' orange fruit and not in lesions of symptomatic fruit.

Regarding the results of the studies presented by Golmohammadi et al. (2007), the authors of the USDA fourth document conclude that it only "*indicate(s) disinfection protocols are not 100 percent effective"*, which is just part of what the authors stated. The results of Golmohammadi et al. (2007) clearly show that: (1) significant parts of *Xanthomonas citri* subsp. *citri* populations do survive shipment, (2) *Xanthomonas citri* subsp. *citri* populations surviving shipment are still infectious as shown by inoculation on susceptible host plants, (3) *Xanthomonas citri* subsp. *citri* populations survive field surveys, culling and packinghouse processes. Golmohammadi et al. (2007) also pointed out the fact that *Xanthomonas citri* subsp. *citri* populations may not be accessible to analysis by plating as a viable but non-culturable state (VBNC) may be induced by shipment conditions, which does not prevent

bacteria from infecting susceptible host plants. Studies indicated that the viable but non-culturable state in *Xanthomonas citri* subsp. *citri* was observed under laboratory conditions (Cubero and Graham, 2002), and that *Xanthomonas citri* subsp. *citri* cells also entered the VBNC state after copper treatment and retained their virulence (Del Campo et al., 2009).

It should also be kept in mind that the citrus fruit analysed by Golmohammadi et al. (2007), were declared to be in conformity with the EU requirements, as they originated from orchards inspected and declared healthy, and being isolated from any contaminated orchards, and that the harvested citrus fruit were culled, cleaned, disinfected and shipped refrigerated. Even when complying with those EU import requirements, a large number of interceptions by the EU Member States has been recorded (see Appendix B, Table 1). All those interceptions were made on the basis of primary visual inspections of fruit.

Amongst others, the following weaknesses were pointed out in the previous EFSA opinion (EFSA, 2006):

- even though fruit are produced under strict conditions, interceptions occur for instance when imported to Europe, which basically proves that *Xanthomonas citri* subsp. *citri* is perfectly able to escape field survey, culling at harvest and packinghouse processes,

- the decline in bacterial populations from harvest to consumption does not imply that *Xanthomonas citri* subsp. *citri* cannot survive to shipment.

These weaknesses are still not properly addressed in the USDA second document (USDA, 2007a) and therefore remain valid.

The EFSA PLH Panel considers that significant populations of *Xanthomonas citri* subsp. *citri* may survive shipment conditions. Moreover, the surviving quantities of inoculum per fruit consignment are now even increased in the context of management option 2 retained by the USDA in its rules and regulations (USDA/APHIS, 2009).

4.1.6. Event 4: fruit with inoculum goes to an area with conditions suitable for infection

Conclusions of the USDA second document (USDA, 2007a) regarding event 4.
- Although shipment of imported and domestically grown infected fruit to a suitable habitat is possible, the fraction that would be shipped to a suitable habitat is small. The fraction that would reach a suitable host is smaller.

The USDA fourth document (USDA, 2009a) [as well as the USDA third document (USDA, 2008)] just mentions that 'no new information' is available which could change the conclusions laid down in the USDA second document.

In the USDA second document, based on conclusions attributed to Borchert et al.(2007), it is assumed that "*only a relatively limited proportion of the citrus growing areas in the United States are at risk, as suitable conditions for the disease occur mainly in Florida"*. This is not what is stated in Borchert et al. (2007) paper, which only points out that *"The climate in Florida is highly favourable for citrus canker disease development in terms of predicted spread events, number of favourable days for infection, and average monthly temperatures. The Louisiana and Texas citrus-growing areas have spread events, favourable days for infection and favourable monthly temperatures conducive for moderate to high citrus canker disease intensity. Conditions in the Louisiana are more conducive than in Texas. The Arizona citrus-growing area has monthly temperatures conducive for Xac*

infection, but low annual precipitation, low numbers of favourable days for infection, and few spread events, which result in low potential disease intensity. The California citrus-growing areas have fewer months of temperature conducive for Xac infection than the other citrus-growing areas in the United States. California also had the lowest number of spread events and favourable days for infection with the exception of Arizona. In California, these events occur predominantly during the winter, while warmer summer months are dry, accounting for less than 5% of the annual precipitation."

The EFSA PLH Panel considers that, even if it is true that suitable conditions for citrus canker occur in Florida, and partly explain the epidemics there, conducive conditions are also found elsewhere. Citrus canker occurred for instance in six additional counties, mainly of the Gulf Coast States, during the first outbreak of the disease in the United States (Schoulties et al., 1985; Dopson, 1964). Even though the disease at that time was less serious in those States, it was recognized as a threat for the existence of the citrus industry of the Gulf Coast States (Berger, 1914; Dopson, 1964). An eradication campaign was conducted in all these States, and Texas was the last to fully achieve it.

In addition, Borchert et al. (2007) paper does not take into account the irrigation of citrus orchards, which alters the conditions in the citrus canopy in a way more conducive for citrus canker disease. Even under dry weather conditions, irrigation may lead to local humid conditions in the canopy, favourable for bacterial establishment and disease development. The paper by Vicent and Garcia-Jimenez (2008) demonstrates that in Spain, due to the formation of dew, rainfall and rain days were not positively correlated with citrus canopy wetness. More information on the effects of the microclimate on the epidemiology of citrus canker is needed for accurate estimation of the risks. This was confirmed by the recent reports of citrus canker outbreaks in sub-Saharan regions in the African continent in East-Africa, Somalia (Balestra et al., 2008) and Ethiopia (Derso et al., 2009), and in Western Africa, Mali (Balestra et al., 2008; Derso et al., 2009; Traoré et al., 2008). The high incidence reported on lime in Ethiopia (as much as 80%) and Mali (up to 50%), where a long dry season persists, is indicative that citrus canker can establish and develop in areas with climatic conditions which in the past were not considered to be suitable for the disease.

Currently, all EU Member States import citrus fruit from Third Countries, including countries infested with citrus canker. When cleared from customs, fruit can circulate freely throughout the EU. According to the EU regulation, goods lawfully imported through whichever border inspection point can later freely circulate throughout the EU, without any further inspection. It means that a citrus fruit consignment imported into the EU by a non citrus-growing Member State can finally arrive at a Member State where citrus is a crop of importance, and this final destination is not necessarily known when the clearing from customs takes place.

The EFSA PLH Panel also notes that a significant quantity of citrus fruit imported into the EU enters citrus-growing Member States, when the local produce is not available. Therefore, the EFSA PLH Panel considers that fruit carrying inoculum may go to EU areas with conditions suitable for infection.

In the previous EFSA opinion (EFSA, 2006), detailed criticism was made, which led the PLH Panel to conclude in particular that "*the analysis presented (in the USDA first document, USDA, 2006) is insufficiently detailed to apply to other countries (...)*". For instance, it was pointed out that no objective description of a suitable climate was given and that *Xanthomonas citri* subsp. *citri* may also establish in residential areas (private or public gardens, *etc*) or in citrus nurseries.

USDA did not take into account that criticism, either in the USDA third (USDA, 2008) or fourth (USDA, 2009a) document.

The EFSA PLH Panel concludes that fruit with *Xanthomonas citri* subsp. *citri* inoculum may go to areas with climatic conditions suitable for infection. Such conditions are not as rare as described by the

USDA (USDA, 2009a). Due to (i) the importation of citrus fruit by all EU Member States, including those producing citrus, and (ii) the free circulation of plants and plant products throughout the EU, a significant quantity of citrus fruit imported into the EU may go to citrus-growing areas.

4.1.7. Event 5: inoculum encounters a suitable host and conditions for disease development

Conclusions of the USDA second document (USDA, 2007a) regarding event 5.
- It is unlikely that viable bacteria from an infected fruit would encounter a suitable host under the conditions required for disease development.

In the USDA third and fourth documents (USDA, 2008; 2009a), "*conditions for disease development*" refer only to climatic conditions. Nevertheless, and even if entry and establishment are prerequisites, other factors may also positively influence disease establishment and spread, such as susceptibility of citrus species and cultivars, pruning, fertilization, irrigation and other cultural practices applied in orchards as well as the presence of the Asian citrus leaf miner, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae).

The existence of semi-managed or unmanaged host plants in the citrus-producing areas is also a favourable factor, as citrus species grown in private/public gardens for family consumption or as ornamentals may act as reservoirs of inoculum (Civerolo, 1984). Citrus canker can persist on a tree and inoculum can be amplified when humidity, temperature and susceptibility growth stage of the plant tissues are combined. A specific climatic event (not always extreme) can initiate the dispersal and produce an epidemic in groves (Gottwald et al., 1992).

Xanthomonas citri subsp. c*itri* can grow *in vitro* in a range of about 5 to 37°C. Citrus canker may develop at temperatures between 14 and 36°C, with an optimum around 30°C, whereas, free moisture on the surface of citrus tissues is necessary for the bacterial spread (Civerolo, 1984: Stall and Seymour, 1983).

Epiphytic populations of *Xanthomonas citri* subsp. *citri* recovered from leaves fluctuated during the day and were generally higher early in the morning in the presence of dew (Timmer et al., 1996). The recovery from symptomatic leaves also fluctuated throughout the year and populations recovered seemed to decrease in June-July (winter time in Argentina) (Timmer et al., 1996). In Argentina, *Xanthomonas citri* subsp. *citri* natural populations in fruit lesions did not strongly fluctuate as the lesions aged until the lesions overwintered and then populations decreased about 100 fold (Stall et al., 1980). This decrease can be dramatic through the winter season in Japan (Koizumi, 1977). Thus a discontinuity in the *Xanthomonas citri* subsp. *citri* populations is present in regions where there is a marked winter season. When the winter temperatures are milder, as in a tropical environment, *Xanthomonas citri* subsp. *citri* populations were not strongly affected and decreased approximately 10 fold (Pruvost et al., 2002).

The USDA concludes that commercially produced asymptomatic and symptomatic citrus fruit are not a likely pathway for the transmission of *Xanthomonas citri* subsp. *citri* in the natural environment. With respect to the asymptomatic fruit, the PLH Panel in its previous opinion (EFSA, 2006) provided detailed criticism on the USDA first document (USDA, 2006). For instance, the role of irrigation in the splash dispersal of the bacteria should have been taken into consideration. Due to multiple leaf flushing and fruiting periods, different citrus species and cultivars have long periods of susceptibility, nursery plants are especially susceptible, and the presence of the Asian citrus leaf miner makes citrus susceptible independently of their genetic resistance to the bacterium. USDA did not take that criticism into consideration, neither in the USDA second document (USDA, 2007a) nor in the USDA fourth

document (USDA, 2009a). With respect to symptomatic fruit, as bacterial loads are higher, the risk is higher than the one related to asymptomatic fruit.

The USDA fourth document (USDA, 2009a) recalls that the USDA second document (USDA, 2007a) stated "*it is unlikely viable bacteria from an infected fruit would encounter a suitable host under the conditions required for disease development"*. In case of symptomatic fruit and under conducive conditions for citrus canker development, the risk of infection is even higher than the one with asymptomatic fruit because the inoculum load is higher.

The authors of the USDA fourth document (USDA, 2009a) conclude that "*(…) asymptomatic and symptomatic fruit produced commercially are not a likely pathway for the transmission of Xcc in the natural environment*". But at the same time, they recognise that under an experiment with only a limited number of replicates, *"Xcc developed a single lesion on a leaf of a susceptible tree in a wound site, from Xcc bacteria transmitted from the fruit"*. The EFSA PLH Panel notes that these two statements are mutually incompatible.

The authors of the USDA fourth document (USDA, 2009a) also assert that "*these results support years of empirical data indicating that mature commercially produced citrus fruit are not a pathway for the transmission of the pathogen under most conditions likely to be encountered. The movement of citrus fruit has not been associated with an outbreak of the disease anywhere in the world*". Such a conclusion is a pure speculation as it is not based on any scientifically sound evidence. Objectively, the only conclusion which can be drawn is that, to date, no scientifically sound evidence either validates or invalidates that contaminated or infected fruit has been responsible of an outbreak of citrus canker in the past.

Moreover, as the USDA retains the management option 2 described in its rules and regulations for export of citrus fruit to the EU, the number of cases where imported contaminated or infected fruit may be in the vicinity of susceptible citrus trees would necessarily increase.

The EFSA PLH Panel concludes that suitable host plants are present within the EU. It also concludes that the risk is increased in the case of asymptomatic citrus fruit originating from contaminated/infested orchards, and it is even higher in the case of symptomatic fruit.

4.1.8. 'Uncertainties' given by the USDA fourth document

The term 'uncertainty' used by the authors of the USDA documents is not equivalent to that defined in ISPM No. 11. The former consider 'uncertainty' as a question not yet answered by the science (*e.g."can symptomatic fruit (…) treated (…) transmit the bacteria (…)*") and which then needs further research, whereas the latter defines it as the level of confidence reached in the ranking of an event important in the PRA scheme (*e.g.* probability a pest can enter an area…), on the basis of available reliable pieces of information.

In their USDA second document (USDA, 2007a), the authors listed six [and not five as stated in the USDA fourth document (USDA, 2009a)] "*key research needs that would strengthen regulatory decision making"*. Three out of the six research needs are addressed in the USDA fourth document (USDA, 2009a). The following issues, despite being mentioned in the USDA second document (USDA, 2007a), are not addressed: (1) "*what is the relationship between the proportion of symptomatic fruit in the field and the proportion of infected fruit after post harvest culling"*, (2) "*what is the efficiency of specific packinghouse equipment and procedures in removing blemish fruit"*, and (3) "*how effective are quality assurance and oversight systems*".

Regarding 'uncertainties' in the sense of ISPM No.11 the authors of the USDA documents do not provide any factual information. Instead they make assertions such as for instance (USDA, 2009a) "*asymptomatic fruit (…) is not epidemiologically significant"* or *"present(s) a low risk of introducing citrus canker*" (pages 2, 3, 5, 12 and 13, USDA, 2009a).

4.1.9. Conclusion of the USDA fourth document

As argued in previous parts of this opinion dedicated to the review of papers from Gottwald et al. (2009) and Shiotani et al. (2009), the EFSA PLH Panel cannot support the USDA conclusion stating that field experiments demonstrated that transmission of *Xanthomonas citri* subsp. *citri* from citrus fruit to the natural environment is not possible under normal conditions.

The authors of the USDA fourth document (USDA, 2009a) inserted in their conclusion speculations which were neither presented nor discussed previously in their documents: *"empirical data from (…) interceptions demonstrates that even with a high frequency of unauthorised citrus fruits imports, outbreaks linked to fruit have never been observed. Several years of shipments (…) from countries where Xcc occurs (like Argentina) to suitable locations (like Europe) have occurred without disease spread associated with the movement of this fruit. This empirical data further inform the uncertainties about epidemiology".*

The EFSA PLH Panel acknowledges that the USDA documents do recognise that Europe is a suitable location for the establishment of citrus canker.

Nevertheless, the EFSA PLH Panel notes that imports have to comply with the EU phytosanitary regulation (refer to consolidated Directive 2000/29/CE, annex IV, part A, chapter 1, especially article 16.2), which in simple terms does not allow import of fruit from fields (or their immediate vicinity) where symptoms of citrus canker were observed during the growing season. Nevertheless, at least eight interceptions of citrus fruit originating from Argentina and showing symptoms of citrus canker, were made by the EU Member States inspection services, during the period 2001-2004 and three additional interceptions were made in 2009 (see Appendix B, Table 1). This means that, despite the strict controls made by Argentinean plant health authorities (Canteros, 2004) to comply with EU regulation, symptomatic fruit escaped. The PLH Panel also considers that, should the controls be relaxed, the frequency of symptomatic fruit in consignments imported into the EU from infested areas would dramatically increase.

The authors of the USDA fourth document (USDA, 2009a) again inserted in their conclusion new elements, such as: "*A requirement for minimal presence of lesions on fruit may be justifiable only when typical packinghouse process are unavailable or when the movement of symptomatic fruit to suitable areas (…) within 24 hours of harvest are highly likely to occur*". Such a procedure would considerably increase the level of risk.

4.1.10. Executive summary of the USDA fourth document

The Executive summary starts by bringing together the conclusions the authors made in the USDA fourth document (USDA, 2009a), on which the EFSA PLH Panel has already commented.

Nevertheless, the authors of the USDA fourth document (USDA, 2009a) also bring new statements in the last paragraph of the Executive summary, not supported at all in the core text. The sentence "*when typical packinghouse processes are unavailable*" suggests that derogations to constraints may apply, or would apply, for packing stations. This statement is not supported by any of the scientific documents provided by USDA. No information is given on what can be a 'non typical' packinghouse process.

It is also suggested that minimizing the presence of lesions in export fruit lots would only be justified when "*movement of symptomatic fruit to suitable areas (…) (would be highly likely to occur) within 24 hours of harvest*". This statement is not supported by any of the provided or discussed documents. This implies that no check at all would be done when exporting fruit from infested areas and orchards, providing that a delay of only 24 hours post-harvest is ensured. This is a major deviation not supported by any scientific document.

4.1.11. Conclusion of the EFSA PLH Panel on the USDA-APHIS document 'Updated evaluation of citrus fruit (Citrus spp.) as a pathway for the introduction of citrus canker disease (Xanthomonas citri subsp. citri)', version May 2009

The EFSA PLH Panel recalls that most of the weaknesses of the USDA first document (USDA, 2006) pointed out in its previous opinion (EFSA, 2006) have not been adequately taken into consideration in the subsequent documents produced by USDA-APHIS (USDA, 2007a, 2008, 2009a) and therefore remain largely unanswered.

The new pieces of scientific information, which, according to the USDA fourth document (USDA, 2009a), are provided by the papers from Gottwald et al. (2009) and Shiotani et al. (2009), are not conclusive (see previous sections of this opinion). Therefore, the EFSA PLH Panel concludes that its previous scientific opinion (EFSA, 2006) is still valid.

In the last paragraph of the Executive summary (USDA, 2009a), the USDA proposes that, in case typical packinghouse processes are unavailable or when the movement of symptomatic fruit to suitable areas occurs within 24 hours of harvest, the risk of introducing *Xanthomonas citri* subsp. *citri* is reduced only by minimizing the number of symptomatic fruit. This is not supported by any of the information provided by the USDA documents.

After analysing the two USDA documents (USDA, 2009a, b), the EFSA PLH Panel concludes that:

- it is likely that, when citrus fruit are permitted for export from areas infested with *Xanthomonas citri* subsp. *citri*, infected fruit do enter into commerce. Moreover, this probability is now increased in the context of management option 2 retained by the USDA in its rules and regulation.

- significant populations of *Xanthomonas citri* subsp. *citri* can survive packinghouse processes. Moreover, the surviving quantities of inoculum per lot of citrus fruit are increased in the context of management option 2 retained by the USDA in its rules and regulation.

- significant populations of *Xanthomonas citri* subsp. *citri* can survive shipment conditions. Moreover, the surviving quantities of inoculum per lot are now even increased in the context of management option 2 retained by the USDA in its rules and regulation.

- fruit with *Xanthomonas citri* subsp. *citri* inoculum may go to areas with climatic conditions suitable for infection. Such conditions are not as rare as described by the USDA (USDA, 2009a). Due to (i) the importation of citrus fruit by all Member States, including citrus-producing ones, and (ii) the free circulation of plants and plant products throughout the EU, a significant quantity of citrus fruit imported into the EU may enter citrus-growing areas.

- suitable host plants are present within the EU citrus-producing Member States.

- the risk occurs in the case of asymptomatic citrus fruit originating from infested orchards, and it is even higher in the case of symptomatic fruit.

4.2. Scientific opinion on the USDA-APHIS document 'Supplemental risk management analysis of movement of commercially packed citrus fruit from citrus canker disease quarantine area', version dated May 2009.

For more clarity, this part of the opinion is organised according to the structure of the given USDA document, except for comments on the Executive summary which are postponed to the end. In addition, some background information is added at the beginning of this Section.

4.2.1. Background information

In May 2009, USDA-APHIS produced a document entitled 'Supplemental risk management analysis; movement of commercially packed citrus fruit from citrus canker disease quarantine area' (USDA, 2009b). This document, which was submitted to the EFSA PLH Panel for scientific evaluation, is hereafter referred to as 'USDA final sRMA document'. As this document originates from a series of previously published USDA-APHIS documents, its analysis requests some input from all those documents.

In June 2007, USDA-APHIS issued the first document called 'Risk management analysis, movement of commercially packed citrus fruit from citrus canker disease quarantine area' (USDA, 2007b). This document is hereafter referred to as the 'USDA first RMA document'. It describes in particular the five different sets of risk management options envisaged by USDA-APHIS at that time, for commercially packed fruit.

In September 2007, a revised version of the USDA first RMA document called 'Revised risk management analysis, movement of commercially packed citrus fruit from citrus canker disease quarantine area' was issued (USDA, 2007c). That document is hereafter referred to as the 'USDA rRMA document'. This document had been peer-reviewed by three reviewers whose reports are included in another USDA document entitled 'Citrus canker peer review, final report' (USDA, 2007d) and published in November 2007.

In March 2009, USDA-APHIS published a new document (USDA, 2009e) called 'Supplemental risk management analysis for movement of commercially packed citrus fruit from citrus canker disease quarantine area'. That document is an update of the USDA rRMA document (USDA, 2007b) for the sections pertaining to the biology and epidemiology of citrus canker and is hereafter referred to as 'USDA sRMA document'. This document had been peer-reviewed by three other reviewers working independently from each other and from USDA, as specified in the Office of Management and Budget guidelines (U.S. Office of Management and Budget, 2004). Reviewers were asked to answer a series of seven pre-defined questions related to the USDA sRMA document. In the same month (March 2009), USDA issued a document called 'Peer review of supplemental risk management analysis for movement of citrus fruit from citrus canker disease quarantine area, final report' (USDA, 2009c). This document includes the scope of the review, the tasks attributed to the reviewers and the reviewers' reports.

In June 2009, USDA published a 'Response to peer review of the supplemental risk management analysis, movement of citrus fruit from citrus canker disease quarantine area' (USDA, 2009d) (hereafter referred to as the 'Response to peer review of the USDA sRMA document'). This document was apparently published after the 'USDA final sRMA document' (USDA, 2009b), which was submitted to the EFSA PLH Panel for scientific evaluation. This document reflects the USDA point of view on the responses given and the conclusions drawn by the three reviewers. USDA mentions in the 'Response to peer review of the sRMA document' that it took care to improve the sRMA. Nevertheless, the PLH Panel notes that no indications are given on how improvements were made.

Although all the above-mentioned documents have been prepared after 2006, when the scientific opinion of the EFSA's PLH Panel (EFSA, 2006) on the USDA document 'Evaluation of **a**symptomatic citrus fruit (*Citrus* spp.) as a pathway for the introduction of citrus canker disease (*Xanthomonas axonopodis* pv. *citri*)' (USDA, 2006) was published, no reference is made by the authors of the USDA documents to the previous EFSA opinion (EFSA, 2006).

4.2.2. Introduction of the USDA sRMA document

The authors of the USDA sRMA document explain that rules laid down in the Federal Register of 2006 (USDA/APHIS, 2006) related to the U.S. interstate movement of citrus and which had been amended in November 2007 (USDA/APHIS, 2007) to allow the movement of fresh fruit under certain conditions. Nevertheless, and among other rules, fruit originated from infested States were still prohibited from distribution to USA commercial citrus-producing States and Territories.

Those rules also stated that "*if, in the future, evidence is developed to support a determination that commercially packed citrus fruit (both asymptomatic and symptomatic) is not an epidemiologically significant pathway for the introduction and spread of citrus canker, (USDA) would undertake rulemaking to amend (US) regulations accordingly"*.

After issuing the USDA fourth document (USDA, 2009a), which refers to the movement of asymptomatic as well as symptomatic fruit as viable pathways for the introduction and spread of citrus canker, and based on two recently published scientific papers (*i.e.* Gottwald et al., 2009; Shiotani et al., 2009), which according to the authors provide relevant new findings, USDA reviewed the corresponding risk management measures.

4.2.3. Purpose and scope of the USDA sRMA document

4.2.3.1. Purpose

The authors of the USDA sRMA document state that "*this document is not intended to either describe the rulemaking/decision making process or any decision reached but rather to evaluate the scientific and technical conclusions of previous analytical documents (USDA, 2007a; b) in light of new information"*. They further state that the purpose of this document is: *"(i) to provide APHIS decision makers with an evaluation of the impact of new evidence on the potential role of commercially packed and disinfected citrus fruit from citrus canker disease quarantine areas in spreading and establishing the citrus canker pathogen to areas previously free of that disease, and (ii) to develop a range of management options to be considered for revisions to APHIS regulations on the movement of fruit from regions quarantined for citrus canker disease based on this analysis as well as separate environmental and economic analyses".*

4.2.3.2. Uncertainties

The USDA second document (USDA, 2007a) and USDA RMA first document (USDA, 2007b), cited by USDA-APHIS in the USDA sRMA document (USDA, 2009b), included the following six *"key uncertainties around the epidemiological significance (or lack thereof) of Xcc associated with symptomatic and asymptomatic commercially packed citrus:*

- *Can symptomatic fruit that has been treated (with SOPP, chlorine, or other appropriate disinfectant) transmit the bacteria that cause the disease (i.e. can disease be incited on*

healthy trees or seedlings from infected, symptomatic fruit that has been treated post-harvest)?

- *How effective are different products at reducing the biological activity of bacteria in lesions (i.e., what is the efficacy of various post-harvest treatments (e.g. SOPP, chlorine, etc) at rendering symptomatic fruit epidemiologically insignificant)?*
- *How long after post-harvest treatment can Xcc be recovered from asymptomatic fruit?*
- *What is the relationship between the proportion of symptomatic fruit in the field and the proportion of infected fruit after post-harvest culling?*
- *What is the efficacy of specific packinghouse equipment and procedures in removing blemished fruit?*
- *How effective are quality assurance and oversight systems?"*

Of the above-mentioned uncertainties, the USDA fourth document (USDA, 2009a) and the USDA sRMA document (USDA, 2009b) retained the first three. However, a fourth uncertainty was added to the USDA sRMA document:

- *"Can wounds on harvested fruit serve as prolonged sources of inoculum for Xcc infection?"*

The three uncertainties, which were not been taken into consideration in the USDA sRMA document (USDA, 2009b), as they were considered by the USDA to be resolved based on the results of studies by Gottwald et al. (2009) and Shiotani et al. (2009), are:

(1) *"what is the relationship between the proportion of symptomatic fruit in the field and the proportion of infected fruit after post harvest culling",*

(2) *"what is the efficacy of specific packinghouse equipment and procedures in removing blemished fruit", and*

(3) *"how effective are quality assurance and oversight systems".*

The EFSA PLH Panel considers that the results of the new research conducted by Gottwald et al. (2009) and Shiotani et al. (2009) do not resolve these uncertainties (see sections 3.1. and 3.2.). Moreover, two additional papers (*i.e.* Christiano et al., 2007, and Golmohammadi et al., 2007), cited for the first time in the USDA sRMA document (USDA 2009b), do not resolve any of these uncertainties either. The paper from Golmohammadi et al. (2007) describes a diagnostic method (isolation and real-time PCR assay) for the reliable detection of *Xanthomonas citri* subsp. *citri* in lesions on processed citrus fruit consignments (see section 3.2.4.). The authors concluded that the compounds recommended for the disinfection of citrus fruit in the packinghouse before exportation are not always sufficient to eliminate viable bacteria and that the presence of such living bacteria constitutes a risk of dissemination of citrus canker through contaminated symptomatic fruit. The paper from Christiano et al. (2007) does not help resolving these uncertainties either, as it deals with the "*effect of citrus leaf-miner damage, mechanical damage and inoculum concentration on severity of symptoms of Asiatic citrus canker in Tahiti lime*" in Brazil. Christiano et al. (2007) showed that the introduction of the leaf-miner, *Phyllocnistis citrella*, in Brazil increased the number of disease foci and modified the spatial pattern of diseased trees from strong aggregation to intermediate aggregation and random patterns. Moreover, the minimum inoculum concentration necessary to cause symptom development was 100 times lower in the presence of the leaf-miner.

4.2.3.3. Assumptions

USDA-APHIS also made the following six assumptions in conducting the sRMA document (USDA, 2009b):

(1) *"The subject studies that prompted this analysis used citrus cultivars that represented the extremes of susceptibility from highly susceptible (grapefruit) to less susceptible varieties (lemon, mandarins). APHIS assumes cultivars not specifically studied would fall within this range of susceptibility and the results are therefore applicable to all citrus cultivars.*

(2) *The fruit that will be affected by the rule is intended for consumption. Fruit or fruit parts that are not consumed are discarded by consumers following standard disposal practices. These practices include placing in the trash intended for landfills, placing in compost heaps or flushing through trash disposal units.*

(3) *Vectors do not have a role in disease epidemiology and if they do, it is not subject to regulation (e.g. long distance dispersal of viable inoculum by birds).*

(4) *Phytosanitary practices are not assumed to be 100% effective but, in addition the measures required by regulation, other practices routinely employed in producing, packing and/or distributing commercially packed citrus, including the time it takes to complete the process from packinghouse to consumer, may further reduce the epidemiological significance of infected fruit.*

(5) *Risk of introduction of citrus canker into other citrus-producing states via the movement of commercially packed citrus from citrus canker quarantine areas is not assumed to be zero.*

(6) *We assume that previous Agency experience with successful prevention and safeguarding informs the likelihood of success of future actions."*

The PLH Panel considers that:

- the second assumption has not taken into account the waste derived from packinghouses. Citrus packinghouses are usually located within citrus-growing areas and waste management does not address the related phytosanitary risks, at least within the EU. In addition, the "*standard disposal practices*" applied in the USA are not described and moreover, they are not necessarily those occurring elsewhere in the world.

- the third assumption refers to a part of citrus canker epidemiology that it is not clearly understood, as there are no studies on the role of vectors, such as animals, insects, birds, etc in the dissemination of *Xanthomonas citri* subsp. *citri*.

- the fourth assumption does not provide any detail with respect to the "*other practices employed in producing, packing and/or distributing packed citrus"* and it appears that these practices are always applied.

- the sixth assumption is scientifically irrelevant and contrasts with the decision made by APHIS (see first paragraph of the Executive summary) to halt citrus canker disease eradication efforts and declare the entire State of Florida a quarantine area.

4.2.3.4. Scope

According to the authors, the scope of the USDA sRMA document (USDA, 2009b) is limited to the pathway "*domestic interstate movement of commercially packed and disinfected fresh fruit from areas where the disease occurs to areas where the disease does not occur".*

The PLH Panel considers that this is contrary to the last paragraph of the Executive summary of the risk evaluation (USDA, 2009a) which implies that fruit may be moved / exported from infested areas where "*typical packinghouse processes are unavailable or when the movement of fruit (…) within 24 hours of harvest are highly likely to occur*". It also contradicts the management option 1 (see below) which does not require any phytosanitary treatment of the fruit in packinghouses.

The authors mention that analysis of the social, environmental and economic consequences are out of the scope of the document, but are nevertheless covered by 'separate analyses'. However, those analyses were not provided or cited.

4.2.4. The movement of commercially packed and disinfected fresh citrus fruit as a pathway for the introduction of Xanthomonas citri subsp. citri

The authors of the USDA sRMA document (USDA, 2009b) consider that "*previous analyses (Schubert et al., 1999; USDA, 1995; 2007a,b) concluded that the likelihood of introducing Xcc into citrus canker disease-free areas on commercially produced and packed citrus fruit is low for the following five reasons:*

(1) Fresh fruit is produced and harvested using techniques that reduce the prevalence of Xcc-infected fruit.

(2) Symptomatic fruit are culled and all fruit are treated for epiphytic contamination by Xcc with disinfectants during commercial packing.

(3) The mortality of Xcc associated with fresh citrus fruit and/or packing materials that occurs following harvest and packing.

(4) For a successful Xcc infection that results in disease outbreaks an unlikely sequence of epidemiological events would have to occur.

(5) Large quantities of fresh citrus fruit have been shipped for many years from regions with Xcc to areas free of the pathogen without any reports of disease outbreaks linked to fresh fruit."

The EFSA PLH Panel considers that the authors of the USDA sRMA document (USDA, 2009b) simply disregarded the arguments related to the above-mentioned five points that had been made in the previous EFSA opinion (EFSA, 2006) and which remain valid. In addition, the EFSA PLH Panel notes that the conclusions drawn by the cited analyses were limited to asymptomatic fruit and thus, they cannot be extrapolated to symptomatic fruit.

In the following five sections, the USDA sRMA document (USDA, 2009b) refers to interpretations of the scientific data originating mainly from the Gottwald et al. (2009) and Shiotani et al. (2009) papers and which, according to the USDA, support the above-mentioned five reasons.

The EFSA PLH Panel considers that, as those two papers have already been extensively analysed and evaluated above (see section 3.1. and 3.2..), only the conclusions reached in the USDA sRMA document (USDA, 2009b) will be given in what follows, together with comments where appropriate.

4.2.4.1. Fresh citrus fruit production and harvesting techniques reduce the prevalence of Xanthomonas citri subsp. citri-infected fruit.

Summary of the paragraph 3.1. given by the USDA (USDA, 2009b).
- Disease management practices in the grove reduce, but do not eliminate, Xcc populations.
- Commercially produced fruit harvested in areas where Xcc exists may be visibly infected or the fruit may carry the pathogen either on its surface or in wounds.
- Citrus canker disease development on citrus fruit between harvest and packinghouse, via wounding, for example, is not likely.

The authors here simply repeat information from the risk management analysis (USDA, 2007b).

In order to support the above three summary points, the USDA has included in this section, among other information, the following statement: "*Based on packing line results in Gottwald et al. (2009), fewer Xcc bacteria were reisolated from naturally occurring fruit lesions in August compared to April, reflecting the effect of fruit age (and therefore lesion age) on inoculum. These results are supported by observations from Japan (Shiotani et al., 2009), where artificially inoculated symptomatic and aging Satsuma mandarin fruit developed very low levels of Xcc, with only a small proportion of lesions producing any inoculum".*

The PLH Panel notes that Gottwald et al. (2009) assumed that the decline in the *Xanthomonas citri* subsp. *citri* population on fruit collected in August was related to the fruit (or lesion) age. Nevertheless, no data was given to support it and only pooled data were presented. According to Stall et al. (1980), natural populations of *Xanthomonas citri* subsp. *citri* did not strongly fluctuate as the lesions aged. Pruvost et al. (2002) reported that, in areas with a marked winter season (*e.g.* Argentina and Japan), low temperatures induce a decrease of 10^2 to 10^4 in population sizes in lesions, thus creating a discontinuity in the *Xanthomonas citri* subsp. *citri* life cycle. Based on the above, the EFSA PLH Panel considers that the decline in *Xanthomonas citri* subsp. *citri* population observed in Gottwald et al. (2009) studies might be due to the lower temperatures occurring in Argentina during the winter (June, August) compared to those in autumn (April).

The authors partially report the results from Gottwald et al. (2009) as they stated "*results from packing line experiments for grapefruit and lemon in which washates from symptomatic fruit produced the highest number of citrus canker disease lesions in bioassays for viable Xcc, compared to asymptomatic fruit and mixed asymptomatic and symptomatic fruit*". The above statement is valid for lemon and for the 2006 experiment on grapefruit, but not for the 2007 experiment on grapefruit.In addition, the number of lesions produced by the washates from the mixture of asymptomatic / symptomatic fruit (4:1 ratio) was not significantly higher than that from apparently healthy fruit (grapefruit or lemon) except for lemon when harvested from apparently healthy trees.

Shiotani et al. (2009) studies deal with Sastuma mandarin fruit, a moderately resistant to resistant citrus species, which reacts to *Xanthomonas citri* subsp. *citri* infection differently from the susceptible species. Therefore, the results of their studies cannot be extrapolated to susceptible citrus species (see section 3.1).

4.2.4.2. Commercial citrus fruit packing techniques reduce the prevalence of infected or contaminated fruit.

Summary of the paragraph 3.2. given by the USDA (USDA, 2009b).
- Procedures for cleaning and disinfecting fruit are routinely applied by packinghouses.

- The individual efficacy of each of these procedures for removing or destroying Xcc may not be known in detail, but the effect of packinghouse treatments reduces the prevalence of viable Xcc and therefore the level of inoculum associated with commercially packed and disinfected fresh citrus fruit.
- Packinghouse treatments reduce the prevalence of Xcc and the level of inoculum associated with and disinfected fresh citrus fruit.
- Packinghouse processing that includes prewashing fruit with detergent over brushes followed by a disinfectant treatment further reduces amounts of Xcc inoculum on infected or contaminated fruit.

The authors here simply repeat information from the risk management analysis (USDA, 2007b).

As the PLH Panel has previously noted (EFSA, 2006), the efficacy of disinfectant treatments appears quite variable and does not achieve the eradication claimed by the authors. In some cases, disinfectant treatments only reduced bacterial populations by 77% (Stapleton, 1986). Stapleton (1986) recovered alive bacteria from commercial dip-tank solutions and found that 2.7×10^2 - 2.9×10^3 cfu/cm^2 of epiphytic bacteria survived dip treatment containing chlorine at concentrations above the recommended 200 ppm level. Additionally, bacterial populations were found to survive at chlorine concentrations of 900 ppm, well in excess of the 200 ppm used commercially (Stapleton, 1986).

4.2.4.3. Mortality of Xanthomonas citri subsp. citri associated with fresh citrus fruit and/or packing materials following harvest and packing.

Summary of the paragraph 3.3. given by the USDA (USDA, 2009b).
- The viability of bacteria on fruit and in lesions and wounds diminishes after the fruit is harvested.
- Epiphytic populations of Xcc may aid in pathogen dispersal, but substantial evidence indicates that bacterial populations do not infect mature fruit.
- Evidence indicates that wounds on harvested fruit containing Xcc inoculum do not lead to citrus canker lesion development, and Xcc populations generally decline, although wounds might occasionally retain Xcc populations that decline more slowly.
- The cool temperatures at which citrus fruit are stored and shipped, and duration of storage reduce the ability of Xcc to reproduce and cause infection.

The authors here simply repeat information from the risk assessment (USDA, 2007b).

The authors support their first conclusion by citing the following three references: Belasque Jr. and Rodriquez-Neto (2000), Graham et al. (1992) and Koizumi (1972). The PLH Panel notes that none of the above references supports the conclusion that the viability of bacteria in lesions diminishes after the fruit is harvested, as:

(1) Belasque Jr. and Rodriquez-Neto (2000) is an abstract that refers to the viability of *Xanthomonas citri* subsp. c*itri* bacteria present as contaminants on the surface of spray-inoculated 'Valencia' orange fruit and not in lesions of symptomatic fruit.

(2) Graham et al. (1992) studies refer to the expansion rate of citrus canker lesions on citrus fruit of different growth stages.

(3) Koizumi (1972) showed that the *Xanthomonas citri* subsp. *citri* population in lesions (of the late infection type) on artificially inoculated Satsuma mandarin fruit gradually decreased after harvest, but it could still be detected after 3 and 5 months on fruit inoculated in late September and late August, respectively. These results are in contrast with Shiotani et al. (2009) studies, which suggested that "*the bacteria appear to be short-lived after fruits are detached from the tree*". Moreover, Koizumi (1972) showed that the bacteria could survive up to 2 or 3 months in

lower temperature season in symptomatic Satsuma mandarin peels buried in depths of 10 or 15 cm or placed on the soil surface. However, when the peels were placed at a height of 1.5 m in a field or in a room, bacteria could be detected up to 3.5 months or 6 months, respectively.

Based on the above, the PLH Panel considers that *Xanthomonas citri* subsp. *citri* bacteria may survive in lesions on harvested fruit long enough to spread the disease to new areas.

In the second conclusion, the USDA acknowledges that epiphytic populations of *Xanthomonas citri* subsp. *citri* may aid in pathogen dispersal. However, it further states that substantial evidence indicates that bacterial populations do not infect mature fruit or survive on mature fruit long enough to infect other hosts. For supporting this statement the USDA cites the Shiotani et al. (2009) studies as well as those of Goto (1962; 1969). The PLH Panel has already commented on the studies conducted by Shiotani et al. (2009) (see section 3.1). Goto (1962; 1969) did not deal with survival of epiphytic *Xanthomonas citri* subsp. *citri* bacteria.

The fourth conclusion is not supported by any scientific evidence. The PLH Panel notes that the low temperatures used for the transport and storage of citrus fruit do not allow the multiplication of *Xanthomonas citri* subsp. *citri* bacteria in lesions [*Xanthomonas citri* subsp. *citri* multiplication *in planta* occurs at temperatures 14-36 ºC (Koizumi, 1976)], but they do not affect their survival. The latter is further supported by the numerous interceptions of the pathogen on citrus fruit originated in infested areas and imported into the EU Member States (see (Appendix B, Table 1) and the data provided by Golmohammadi et al. (2007).

4.2.4.4. Environmental and epidemiological conditions for Xanthomonas citri subsp. citri establishment

Summary of the paragraph 3.4. given by the USDA (USDA, 2009b).
- As a condition for successful establishment, Xcc in amounts sufficient to cause infection, must encounter not only an environment with a conducive temperature, relative humidity, moisture, and wind for infection, but also must encounter host plant tissue that is either at a susceptible growth stage or is wounded and then must successfully enter this tissue.
- Despite substantial international trade between Xcc-infected and non-infected countries, there is no authenticated record of movement of diseased fruit or seeds resulting in the introduction of Xcc to new areas.

The authors here simply repeat information from the risk assessments of 2006 (USDA, 2006) and 2009 (USDA, 2009a), which have already been discussed in the first EFSA opinion (EFSA, 2006) and in the present one (see above, section 4.1.).

4.2.4.5. Conclusions and summary of evidence regarding fruit as a pathway for Xanthomonas citri subsp. citri introduction.

In paragraph 3.5, the authors simply summarise the results of the recent studies of Gottwald et al. (2009) and Shiotani et al. (2009) and conclude, based on this evidence and on that included in the risk assessment (USDA, 2007a) and the USDA rRMA document (USDA, 2007c) that "*commercially packed and disinfected fresh citrus fruit is not an epidemiologically significant pathway for the introduction and spread of Xcc"*.

The PLH Panel considers that, as the four above-mentioned documents do not provide scientifically sound evidence to support the above conclusion, its arguments (EFSA, 2006) with respect to the risk assessment conducted by USDA in 2006 remain valid.

4.2.4.6. Conclusions on the USDA sRMA document (USDA, 2009b)

The EFSA PLH Panel notes that the authors of the USDA sRMA document (USDA, 2009b) disregarded the arguments related to the asymptomatic citrus fruit (*Citrus* spp.) as a pathway for the introduction of citrus canker disease (*Xanthomonas citri* subsp. *citri*) into a new area that had been developed in the previous EFSA opinion (EFSA, 2006) and which remain valid. In addition, the EFSA PLH Panel recalls that the conclusions drawn by the analyses cited in EFSA (2006) were limited to asymptomatic fruit and thus, they cannot be extrapolated to symptomatic fruit.

The USDA sRMA document (USDA, 2009b) refers to interpretations of the scientific data originating mainly from the Gottwald et al. (2009) and Shiotani et al. (2009) papers. Those two papers have already been extensively analysed and evaluated above (see section 3.1. and 3.2.). In addition to the conclusions previously drawn in these sections, the EFSA PLH Panel concludes that:

- the decline in *Xanthomonas citri* subsp. *citri* population on fruit, reported by Gottwald et al. (2009), was related to the season of sampling rather than to the fruit (or lesion) age,
- the efficacy of disinfectant treatments appears quite variable and does not achieve the eradication claimed by the authors,
- none of the references cited by the authors showed that *Xanthomonas citri* subsp. *citri* bacteria do not survive in lesions on harvested fruit for a sufficient time to spread the disease to new areas,
- the numerous interceptions of *Xanthomonas citri* subsp. *citri* on citrus fruit originated in infested areas and imported into the EU Member States, and the Golmohammadi et al. (2007) pathogenicity results, are contrary to the authors statement that the storage and shipment conditions reduce the survival of *Xanthomonas citri* subsp. *citri*.

4.2.5. Risk management options of the USDA sRMA document

Five management options are stated to be supported by USDA in its document (USDA, 2009b), but as one of those options has 2 sub-options (see Table below), in effect six management options are given.

Three modalities are taken into consideration in the USDA sRMA document (USDA, 2009b): (1) destination of citrus fruit within the USA, (2) phytosanitary treatment during the packinghouse process, and (3) inspection of fruit in the packinghouses.

Requirements	Management options					
	1	2	3		4	5
			a	b		
Distribution of fruit to citrus-producing States	+	+	-	+	-	-
Mandatory packinghouse treatments	-	+	+	+	+	+
Mandatory inspection in packinghouses	-	-	-	+	-	+

When the EFSA PLH Panel formed its previous opinion (EFSA, 2006), a systems approach was followed by USDA, which included, among others, field inspections, surveys and field treatments. This systems approach was a major contribution to maintaining the phytosanitary status of citrus-producing areas and to guarantee the minimal bacterial load of citrus fruit. This systems approach was abandoned

by USDA in 2007 (USDA/APHIS, 2007), and since then, only packinghouse inspections, fruit treatment during the packinghouse process and prohibition of movement of fruit from quarantined areas to other commercial citrus-producing States were retained.

The above changes result in a dramatic decrease in the confidence one can have on the bacterial load of traded fruit, and in an associated increase in the risk of spread of *Xanthomonas citri* subsp. *citri* through the fruit pathway.

Option 1 basically would allow free movement of commercially packed citrus fruit within the USA, without treatment in packinghouses and without inspection. The authors of the USDA sRMA document (USDA, 2009b) consider only "*that uncertainties remain regarding the epidemiological significance of untreated fruit*". The PLH Panel considers that Gottwald et al. (2009) and Shiotani et al. (2009) studies do not provide scientifically sound evidence that citrus fruit originating from infested areas is not a pathway for the introduction of citrus canker into new areas (see section 3.1 and 3.2.).

Option 2 retains the free interstate movement of commercially packed fruit with no packinghouse inspections by APHIS, but it introduces a mandatory *"packinghouse treatment with APHIS-approved disinfectant"*. According to the authors of the USDA sRMA document, APHIS would determine whether to continue to require the currently approved disinfectant treatments or apply modifications based on recent research. They also identified uncertainties related to the results of Gottwald et al. (2009) on the effectiveness of the prewash treatment in reducing the likelihood of citrus canker introduction. The authors of the USDA sRMA document (USDA, 2009b) also provide some data collected during informal surveys conducted by APHIS on the potential impacts of adding a prewash treatment to APHIS approved disinfection treatments. Based on these data, only a small percentage (6%) of the 134 Florida packinghouses currently have a prewash treatment and an even smaller percentage (4%) use a detergent prewash with mechanical brushes prior to disinfectant treatment.

In addition, option 2 gives the flexibility to citrus growers, harvesters, and packers to implement phytosanitary measures to prevent and control *Xanthomonas citri* subsp. *citri* infection in the fruit they produce, but without any obligation and guidelines.

The EFSA PLH Panel recalls that packinghouse treatments are not fully effective in eliminating *Xanthomonas citri* subsp. *citri* bacteria on fruit harvested from infested areas.

Option 3 allows packinghouse holders operating in States where the disease is present to choose whether they want to have the possibility to export to commercial citrus-producing States or not, and to manage their operations accordingly. In case they want to export to commercial citrus-producing States, phytosanitary inspections by APHIS would occur after disinfection using an APHIS approved packinghouse treatment and commercial packing. However, as no details on the corresponding procedures (*i.e.* phytosanitary inspections, packinghouse treatments) are given, this option cannot ensure that the packinghouse processed fruit will be free of the pathogen.

The statement made by the authors of the USDA sRMA document (USDA, 2009b) that *"a requirement for minimal presence of lesions on fruit (i.e. inspection) may be justifiable only when typical packinghouse processes are unavailable"* is wrongly reported to have been justified in the USDA fourth document (USDA, 2009a).

Option 4 prohibits the distribution of all types and varieties of citrus fruit to US commercial citrus-producing States and retains as mandatory the packinghouse fruit treatment with APHIS-approved disinfectant. Phytosanitary inspections in the packinghouses are not required by this option.

Option 5, which was the rule in force from the end of 2007 to the end of 2009, is similar to option 4 with an additional requirement for mandatory phytosanitary inspections in the packinghouses by APHIS.

4.2.6. Conclusions on the risk management options

Taking into account its previous opinion (EFSA, 2006), the withdrawal of the USDA systems approach, which was in place until 2007, and the above mentioned five management options, the EFSA PLH Panel considers that the flexibility to move/export symptomatic and asymptomatic citrus fruit from infested or non-infested orchards, will result in an increase in the *Xanthomonas citri* subsp. *citri* load of citrus fruit consignments and in a subsequent increase in the probability of spread of citrus canker through the fruit pathway.

4.2.7. Executive summary of the USDA sRMA document

The first paragraph of the Executive summary (USDA, 2009b) clearly explains that previous US rules and regulations regarding citrus canker failed to eradicate citrus canker in Florida, despite the huge efforts made, which led APHIS to declare the entire State of Florida as a quarantine area. This statement shows how serious citrus canker is and how difficult is to eradicate it once it establishes in a new area.

The second paragraph repeats the conclusions of the USDA documents dated 2007 (USDA, 2007a; b) that: "*commercially packed citrus fruit is not an epidemiologically significant pathway for the introduction and spread of citrus canker*". However, in drawing this conclusion, the USDA has not taken into account the conclusions made by the EFSA PLH Panel in its previous opinion (EFSA, 2006).

4.2.8. Conclusions of the EFSA PLH Panel on the USDA-APHIS document 'Supplemental risk management analysis of movement of commercially packed citrus fruit from citrus canker disease quarantine area', version May 2009

The EFSA PLH Panel acknowledges that this document is mainly intended to supplement the previously released RMA document, but its scope is too limited. The EFSA PLH Panel notes that the authors of the USDA sRMA document (USDA, 2009b) disregarded the arguments related to the asymptomatic citrus fruit (*Citrus* spp.) as a pathway for the introduction of citrus canker disease (*Xanthomonas citri* subsp. *citri*) into a new area that had been developed in the previous EFSA opinion (EFSA, 2006) and which remain valid. In addition, the EFSA PLH Panel recalls that the conclusions drawn by the analyses cited in the EFSA (2006) were limited to asymptomatic fruit and thus, they cannot be extrapolated to symptomatic fruit.

The USDA sRMA document (USDA, 2009b) refers to interpretations of the scientific data originating mainly from the Gottwald et al. (2009) and Shiotani et al. (2009) papers. Those two papers have already been extensively analysed and evaluated in the first part of this document (see section 3.1. and 3.2.) and were shown to be not appropriately documented. In addition to the conclusions previously withdrawn in these sections, the EFSA PLH Panel concludes that:

- the decline in *Xanthomonas citri* subsp. *citri* population on fruit reported by Gottwald et al. (2009) was related to the season of sampling rather than to the fruit (or lesion) age,
- the efficacy of disinfectant treatments appears quite variable and does not achieve the eradication claimed by the authors,

- none of the references cited by the authors showed that *Xanthomonas citri* subsp. *citri* bacteria do not survive in lesions on harvested fruit long enough to spread the disease to new areas,

- the numerous interceptions of *Xanthomonas citri* subsp. *citri* on citrus fruit originated in infested areas and imported into the EU Member States and the Golmohammadi et al. (2007) pathogenicity results contradict the authors statement that the storage and shipment conditions reduce the survival of *Xanthomonas citri* subsp. *citri*.

Taking into account its previous opinion (EFSA, 2006), the withdrawal of the USDA systems approach, which was in place until 2007, and the five above-mentioned management options, the EFSA PLH Panel considers that the flexibility to move/export symptomatic and asymptomatic citrus fruit from infested or non-infested orchards, will result in an increase in the *Xanthomonas citri* subsp. *citri* load of citrus fruit consignments and in a subsequent increase in the probability of spread of citrus canker through the fruit pathway.

In addition, the USDA sRMA (USDA, 2009b) does not propose any method to monitor the efficacy of the selected measures, which is a major failure in the decision scheme.

5. Conclusions

After having considered all the evidence, the Panel reached to the following conclusions:

- The EFSA PLH Panel recalls that most of the weaknesses of the USDA first document (USDA, 2006) pointed out in its previous opinion (EFSA, 2006) have not been adequately taken into consideration in the subsequent documents produced by USDA-APHIS (USDA 2007a, 2008, 2009a) and therefore remain largely unanswered.

- The new pieces of scientific information, which, according to the USDA fourth document (USDA, 2009a) are provided by the papers from Gottwald et al. (2009) and Shiotani et al. (2009), are not conclusive. Therefore, the EFSA PLH Panel concludes that its previous scientific opinion (EFSA, 2006) is still valid.

With regard to the review of the scientific paper from Shiotani et al. (2009):

The aim of the paper of Shiotani et al. (2009) was to evaluate the phytosanitary risk to importing countries posed by mature Satsuma mandarin fruit harvested from diseased trees by:

- determining the presence of *Xanthomonas citri* subsp. *citri* on these fruit,

- evaluating the potential transmission of the pathogen from fruit to susceptible hosts.

The PLH Panel, after its review concluded that:

- results from Shiotani et al. (2009) studies, where Satsuma mandarin, a citrus species with two resistance characters (i.e. lesser hyperplasia with little rupture of epidermis and lower bacterial population in the tissue) was used, cannot be extrapolated to susceptible citrus cultivars or species,

- in the experiments on the potential of spread of citrus canker from infected Satsuma mandarin fruit within a sweet orange orchard, no information is provided on the susceptibility of the trees during the experiments and little is given on the prevailing environmental conditions (simultaneous presence of rainfall and susceptible tissues) and agricultural practices (irrigation,

fertilisation *etc.*) applied. The level of inoculum on the experimental fruit was not monitored at the beginning of the experiments,

- methods and procedures used in this paper missed important information to ensure that the detection of *Xanthomonas citri* subsp. *citri* was truly negative in the experiments. Consequently, it is impossible to draw any consistent conclusions from this paper, as: (i) the absence of detection by any of the methods used cannot be interpreted due to the lack of a sensitivity level and positive controls associated with the PCR test, (ii) the method used to recover the bacteria from the samples and the selectivity of the culture medium were not appropriate, and (iii) the level of maturity of the sweet orange leaves used in the bioassays was not appropriate to optimize disease expression as they were mature and thus not fully susceptible.

With so many weaknesses in the detection methods and a citrus species that cannot be considered as a relevant model for citrus canker dispersal, the results of this study cannot be transferred to a more general risk assessment of citrus canker.

With regard to the review of the scientific paper from Gottwald et al. (2009):

The paper of Gottwald et al. (2009) is a compilation of various experiments conducted in Florida and Argentina in order to determine:

(i) the effectiveness of current and modified packinghouse decontamination treatments to reduce the recovery of *Xanthomonas citri* subsp. *citri* from contaminated and infected fruit,

(ii) the epidemiological potential for symptomatic citrus fruit that have passed through the packinghouse undetected to act as a source of inoculum for the infection of susceptible citrus trees in the orchard, and,

(iii) the risk of infection from unprocessed, discarded symptomatic fruit under simulated severe wind-rain conditions.

The PLH Panel, after having critically reviewed the Gottwald et al. (2009) paper, concluded that:

- Occurrence of *Xanthomonas citri* subsp. *citri* on asymptomatic citrus fruit collected in infected orchards is not uncommon, as viable *Xanthomonas citri* subsp. *citri* cells on apparently healthy fruit were detected in some of the experiments.

- The decline observed in the bacterial populations, including those of *Xanthomonas citri* subsp. *citri* after packinghouse treatments, was not statistically significant.

- Chlorine applied at the commercial concentration of 200 ppm with or without prewash and/or detergent did not completely disinfect fruit.

- There was a decrease in the *Xanthomonas citri* subsp. *citri* populations in fruit after harvest, but the number of analysed fruit was not large enough, the variability in their bacterial populations was high and the use of numbers of total bacteria as indicators of *Xanthomonas citri* subsp. *citri* survival, was not accurate.

- The experiments on simulated bacterial dispersal from fruit cull piles and fruit suspended in citrus trees suggest that mature citrus fruit are very poor sources of *Xanthomonas citri* subsp. *citri* inoculum. Despite the fact that the size/architecture of the canopy and the total leaf area of the trap plants exposed to the wind-driven rain were not comparable with those of mature citrus

trees grown in commercial orchards, effective dispersal of *Xanthomonas citri* subsp. *citri* cells did occur, though at a low frequency.

- The experiments on simulated *Xanthomonas citri* subsp. *citri* dispersal were dealing with dispersal by wind-driven rain and not with direct or drip splash dispersal of *Xanthomonas citri* subsp. *citri* cells from symptomatic fruit discarded on the orchard floor. Therefore, the results cannot be extrapolated to a situation where symptomatic fruit/peels have been discarded underneath or in close proximity to susceptible mature citrus trees.

- In many assessments the authors assumed that culturable *Xanthomonas citri* subsp. *citri* cells are the only viable cells ignoring that a viable but non-culturable state (VBNC) of *Xanthomonas citri* subsp. *citri* may also occur. Reliable detection methods (*e.g.* molecular techniques) were not applied to confirm some negative results and to identify *Xanthomonas citri* subsp. *citri*.

- The authors refer most of the time to the results of Shiotani et al. (2009) studies, where the data are not reliable and from which no relevant conclusions can be drawn and ignore the studies of Golmohammadi et al. (2007), which clearly showed that *Xanthomonas citri* subsp. *citri* can survive on packinghouse processed citrus fruit.

With regard to the scientific opinion on the USDA-APHIS 'updated evaluation of citrus fruit (Citrus spp.) as a pathway for the introduction of citrus canker disease (*Xanthomonas citri* subsp. *citri*)', version May 2009:

The new pieces of scientific information, which, according to the USDA fourth document (USDA, 2009a), are provided by the papers from Gottwald et al. (2009) and Shiotani et al. (2009), are not conclusive (see section 3.1 and 3.2). Therefore, the EFSA PLH Panel concludes that its previous scientific opinion (EFSA, 2006) is still valid.

In the last paragraph of the Executive Summary (USDA, 2009a), the USDA brings the idea that, in case typical packinghouse processes are unavailable or when the movement of symptomatic fruit to suitable areas occurs within 24 hours of harvest, the risk of introducing *Xanthomonas citri* subsp. *citri* is reduced only by minimizing the number of symptomatic fruit. This is not supported by any of the information provided by the USDA documents.

After analysing the two provided USDA documents (USDA, 2009a, b), the EFSA PLH Panel concluded that:

- it is likely that, when citrus fruit are permitted for export from areas infested with *Xanthomonas citri* subsp. *citri*, infected fruit do enter into commerce. Moreover, this probability is now increased in the context of management option 2 retained by the USDA in its rules and regulation.

- significant populations of *Xanthomonas citri* subsp. *citri* can survive packinghouse processes. Moreover, the surviving quantities of inoculum per lot of citrus fruit are now even increased in the context of management option 2 retained by the USDA in its rules and regulation.

- significant populations of *Xanthomonas citri* subsp. *citri* can survive shipment conditions. Moreover, the surviving quantities of inoculum per lot are now even increased in the context of management option 2 retained by the USDA in its rules and regulation.

- fruit with *Xanthomonas citri* subsp. *citri* inoculum may go to areas with climatic conditions suitable for infection. Such conditions are not as rare as described by the USDA (USDA, 2009a). Due to (i) the importation of citrus fruit by all EU Member States, including citrus-producing ones, and (ii) the free circulation of plants and plant products throughout the EU, a significant quantity of citrus fruit imported into the EU may enter citrus-growing areas.

- suitable host plants are present within the EU citrus-producing Member States.

- the risk occurs in the case of asymptomatic citrus fruit originating from infested orchards, and it is even higher in the case of symptomatic fruit.

With regard to the scientific opinion on the USDA-APHIS 'supplemental risk management analysis of movement of commercially packed citrus fruit from citrus canker disease quarantine area', version May 2009:

The EFSA PLH Panel acknowledges that this document is mainly intended to supplement the previously released RMA document, but its scope is too limited. The EFSA PLH Panel notices that the authors of the USDA sRMA document (USDA, 2009b) disregarded the arguments related to the movement of fresh citrus fruit that had been developed in the previous EFSA opinion (EFSA, 2006) and which remain still valid. In addition, the EFSA PLH Panel recalls that the conclusions drawn by the cited analyses were limited to asymptomatic fruit and thus, they cannot be extrapolated to symptomatic fruit.

The USDA sRMA document (USDA, 2009b) refers to interpretations of the scientific data originating mainly from the Gottwald et al. (2009) and Shiotani et al. (2009) papers. Those two papers have already been extensively analysed and evaluated in the first part of this document (see section 3.1. and 3.2.) and were shown to be not appropriately documented. In addition to the conclusions previously withdrawn in these sections, the EFSA PLH Panel concludes that:

- the decline in *Xanthomonas citri* subsp. *citri* population on fruit reported by Gottwald et al. (2009) was related to the season of sampling rather than the fruit (or lesion) age,

- the efficacy of disinfectant treatments appears quite variable and does not achieve the eradication claimed by the authors,

- none of the references cited by the authors showed that *Xanthomonas citri* subsp. *citri* bacteria do not survive in lesions on harvested fruit long enough to spread the disease to new areas,

- the numerous interceptions of *Xanthomonas citri* subsp. *citri* on citrus fruit originated in infested areas and imported into the EU Member States, and the Golmohammadi et al. (2007) pathogenicity results, are contrary to the authors statement that the storage and shipment conditions reduce the survival of *Xanthomonas citri* subsp. *citri*.

Taking into account its previous opinion (EFSA, 2006), the withdrawal of the USDA systems approach, which was in place until 2007, and the five management options, the EFSA PLH Panel considers that the flexibility to move/export citrus fruit (symptomatic and asymptomatic), from infested or non-infested orchards, will result in an increase in the *Xanthomonas citri* subsp. *citri* load of citrus fruit consignments and in a subsequent increase in the probability of spread of citrus canker through the fruit pathway.

In addition, the USDA sRMA (USDA, 2009b) does not propose any method to monitor the efficacy of the selected measures, which is a major failure in the decision scheme.

CONCLUSIONS

The EFSA Plant Health Panel concluded in 2006 (EFSA, 2006) that the transmission of *Xanthomonas citri* subsp. *citri* on asymptomatic citrus fruit was more likely when the fruit were collected from infested than from non-infested areas and groves. This conclusion remains still valid as no scientific studies have been conclusive to prove that asymptomatic fruit (treated or untreated) is not epidemiologically significant as a pathway for introducing citrus canker.

Symptomatic fruit carries more *Xanthomonas citri* subsp. *citri* cells than asymptomatic fruit and the disinfectant treatments do not achieve the eradication of *Xanthomonas citri* subsp. *citri*. Management option 2 (*i.e.* "*allow distribution of all types and varieties of commercially packed fruit to all US States, subject to packinghouse treatment with APHIS-approved disinfectant. No packinghouse phytosanitary inspection is required*") selected by USDA (USDA/APHIS, 2009) leads to the free movement throughout the United States of America of citrus fruit (both asymptomatic and symptomatic) originating from citrus canker-infested orchards. The application of management option 2 will result in an increase in the *Xanthomonas citri* subsp. *citri* load of citrus fruit consignments and in a subsequent increase in the probability of spread of citrus canker through the fruit pathway.

Some data provided in the APHIS-USDA documents support that citrus fruit remain a conceptually possible pathway for transmitting and establishing citrus canker disease. The EFSA Plant Health Panel agrees that transmission of *Xanthomonas citri* subsp. *citri* from infected fruit to a susceptible host is rare. But the withdrawal of the current EU requirement that citrus fruit imported into the EU be sourced from groves where no symptoms of citrus canker have been observed in the field of production and in its immediate vicinity since the beginning of the last cycle of vegetation, will increase the probability of introduction of *Xanthomonas citri* subs. *citri* into new areas.

DOCUMENTATION PROVIDED TO EFSA

1. Letter, dated 09.11.2010 with ref. SANCO.E7/RV/svi(2010)792515 from Bernard Van Goethem to C. Geslain-Lanéelle.

2. USDA, APHIS, May 2009, An Updated Evaluation of Citrus Fruit (Citrus spp.) as a Pathway for the Introduction of Citrus Canker Disease (*Xanthomonas citri* subsp.*citri*).

3. USDA, APHIS, May 2009, Movement of Commercially Packed Citrus Fruit from Citrus Canker Disease Quarantine Area, Supplemental Risk Management Analysis.

4. Rules and Regulations, Citrus Canker; Movement of Fruit from Quarantined Areas, 7 CFR Part 301, p.54431-54445 – Federal Register, Vol.74, No.203, 22 October 2009.

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APPENDICES

A. LITERATURE SEARCH PROCESS ON *XANTHOMONAS CITRI* PATHWAYS

OBJECTIVES AND SCOPE

The purpose of this Annex is to present the search process (i.e. search strategy and information sources searched) performed for the opinion, in order to allow reproducibility of the literature search.

More precisely, the literature search focuses on the pathway as described in the USDA report (USDA, 2009a).

(Step 1) Infected/contaminated fruit are harvested;
(Step 2) Inoculum associated with fruit survives the packing/treatment process;
(Step 3) Inoculum associated with fruit survives shipment;
(Step 4) Fruit with inoculum go to an area with conditions suitable for infection; and
(Step 5) Inoculum encounters a suitable host and conditions for disease development.

THE SEARCH FOR RESEARCH STUDIES

1. Restrictions

The only restriction applied was on the year of publication: from 2006 to April 2011 included. No restriction was applied to the type of literature to be retrieved (*e.g.*: search studies, reports, reviews).

2. Information sources searched

For the purpose of this search, the following databases were searched (provider: ISI Web of Knowledge): CAB Abstracts, FSTA, Medline, Web of Science. In addition, Agris and Agricola were also searched.

3. The search strategy (the search terms and their combination)

The search strategy applied was broad and sensitive.

3.1. Steps 1, 4 and 5 of the pathway

Literature concerning steps 1, 4 and 5 of the pathway as described in the USDA reports was search using a single strategy. This strategy was adapted to each database searched by the information specialist of the team.

3.1.1. Source 1: Agris

Agris does not allow complex queries. The following simpler query was then submitted:

(Xanthomonas canker) AND (citrus citri) AND date:[2006 TO 2010]

Note: the OR operator is optional in Agris.

This search retrieved 28 records. Those records were entered manually in the Endnote™ database, one by one, because not export option is available. Some references are incomplete (the "Vancouver citation" option was used for getting the reference) and cannot be completed because no full text is available.

3.1.2. Source 1: Agricola

The system translated the following query:

"suitable condition?" "epidemiological surveys" "disease distribution" "disease prevalence" "disease development" affected asymptomatic contaminat? develop? dispers? expand? host? infect? inoculat? introduction? lesion? outbreak? pathway? spread? symptom? transmi? epidemi?
AND (Xanthomonas canker? cancr?) AND cit?

Into:

(suitable OR condition? OR epidemiological OR surveys OR disease OR distribution OR disease OR prevalence OR disease OR development OR affected OR asymptomatic OR contaminat? OR develop? OR dispers? OR expand? OR host? OR infect? OR inoculat? OR introduction? OR lesion? OR outbreak? OR pathway? OR spread? OR sy)[in Keyword Anywhere] AND (Xanthomonas OR canker? OR cancr?)[in Keyword Anywhere] AND (cit?)[in Keyword Anywhere]

Leading to 846 records.

It was then simplified as follows:

Affect? asymptomatic? contaminat? develop? dispers? distribution? expand? host? infect? inoculat? introduction? lesion? outbreak? pathway? prevalence? spread? symptom? transmi? epidemi?
AND (Xanthomonas canker? cancr?) AND cit?

Translated as follows by the application:

(Affect? OR asymptomatic? OR contaminat? OR develop? OR dispers? OR distribution? OR expand? OR host? OR infect? OR inoculat? OR introduction? OR lesion? OR outbreak? OR pathway? OR prevalence? OR spread? OR symptom? OR transmi? OR epidemi?)[in Keyword Anywhere] AND (Xanthomonas OR canker? OR cancr?)[in Keyword Anywhere] AND (cit?)[in Keyword Anywhere]

This search led to 128 records, all introduced into EndNoteX1™.

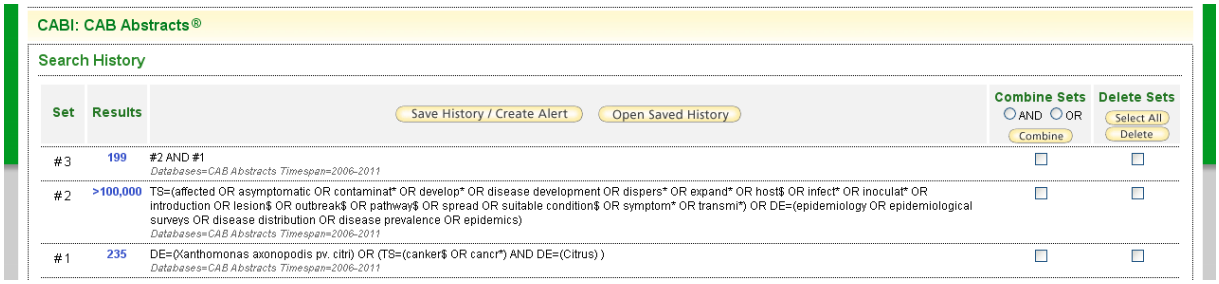
3.1.3. Source 1: CAB Abstracts

Controlled terms are preceded by "DE=":

TS=(affected OR asymptomatic OR contaminat* OR develop* OR disease development OR dispers* OR expand* OR host$ OR infect* OR inoculat* OR introduction OR lesion$ OR outbreak$ OR pathway$ OR spread OR suitable condition$ OR symptom* OR transmi*) OR DE=(epidemiology OR epidemiological surveys OR disease distribution OR disease prevalence OR epidemics) AND DE=(Xanthomonas axonopodis pv. citri) OR (TS=(canker$ OR cancr*) AND DE=(Citrus))

Note: "cancr*" retrieved 1 additional record.

Figure 1: Screenshot of the search realised in CAB Abstracts for the steps 1, 4 and 5 of the pathway



CABI: CAB Abstracts®

Search History

Set	Results	Save History / Create Alert / Open Saved History	Combine Sets (AND / OR, Combine)	Delete Sets (Select All, Delete)
#3	199	#2 AND #1 *Databases=CAB Abstracts Timespan=2006-2011*	☐	☐
#2	>100,000	TS=(affected OR asymptomatic OR contaminat* OR develop* OR disease development OR dispers* OR expand* OR host$ OR infect* OR inoculat* OR introduction OR lesion$ OR outbreak$ OR pathway$ OR spread OR suitable condition$ OR symptom* OR transmi*) OR DE=(epidemiology OR epidemiological surveys OR disease distribution OR disease prevalence OR epidemics) *Databases=CAB Abstracts Timespan=2006-2011*	☐	☐
#1	235	DE=(Xanthomonas axonopodis pv. citri) OR (TS=(canker$ OR cancr*) AND DE=(Citrus)) *Databases=CAB Abstracts Timespan=2006-2011*	☐	☐

3.1.4. Source 2: FSTA

Records concerning Xanthomonas and citrus were retrieved for step 2 (10 records). Thus no additional search was performed.

3.1.5. Source 3: MedLine

Records concerning Xanthomonas and citrus were retrieved for step 2 (69 records). Thus no additional search was performed.

3.1.6. Source 4: Web of Science

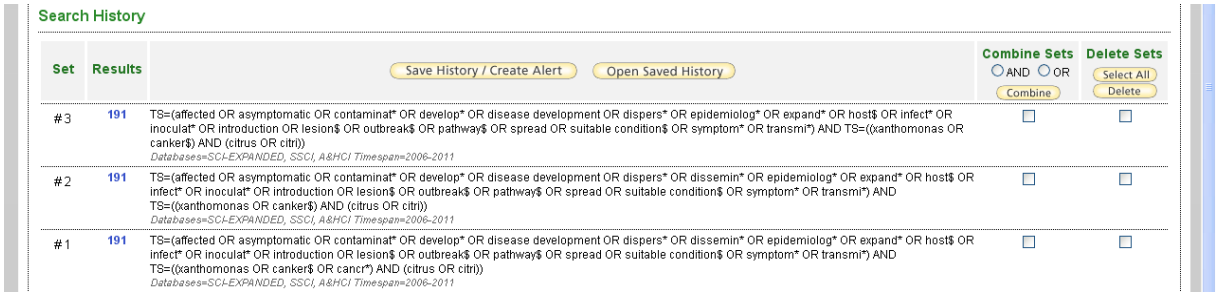
No controlled terms available.

TS=(affected OR asymptomatic OR contaminat* OR develop* OR disease development OR dispers* OR epidemiolog* OR expand* OR host$ OR infect* OR inoculat* OR introduction OR lesion$ OR outbreak$ OR pathway$ OR spread OR suitable condition$ OR symptom* OR transmi*)

AND TS=((xanthomonas OR canker$) AND (citrus OR citri))

Note: adding "OR dissemin*" "OR cancr*" retrieved no additional record.

Figure 2: Screenshot of the search realised in Web of Science for the steps 1, 4 and 5 of the pathway



Search History

Set	Results	Save History / Create Alert / Open Saved History	Combine Sets (AND / OR, Combine)	Delete Sets (Select All, Delete)
#3	191	TS=(affected OR asymptomatic OR contaminat* OR develop* OR disease development OR dispers* OR epidemiolog* OR expand* OR host$ OR infect* OR inoculat* OR introduction OR lesion$ OR outbreak$ OR pathway$ OR spread OR suitable condition$ OR symptom* OR transmi*) AND TS=((xanthomonas OR canker$) AND (citrus OR citri)) *Databases=SCI-EXPANDED, SSCI, A&HCI Timespan=2006-2011*	☐	☐
#2	191	TS=(affected OR asymptomatic OR contaminat* OR develop* OR disease development OR dispers* OR dissemin* OR epidemiolog* OR expand* OR host$ OR infect* OR inoculat* OR introduction OR lesion$ OR outbreak$ OR pathway$ OR spread OR suitable condition$ OR symptom* OR transmi*) AND TS=((xanthomonas OR canker$) AND (citrus OR citri)) *Databases=SCI-EXPANDED, SSCI, A&HCI Timespan=2006-2011*	☐	☐
#1	191	TS=(affected OR asymptomatic OR contaminat* OR develop* OR disease development OR dispers* OR dissemin* OR epidemiolog* OR expand* OR host$ OR infect* OR inoculat* OR introduction OR lesion$ OR outbreak$ OR pathway$ OR spread OR suitable condition$ OR symptom* OR transmi*) AND TS=((xanthomonas OR canker$ OR cancr*) AND (citrus OR citri)) *Databases=SCI-EXPANDED, SSCI, A&HCI Timespan=2006-2011*	☐	☐

3.2. Step 2: Inoculum associated with fruit survives the packing/treatment process

3.2.1. Source 1: Agris

Records concerning Xanthomonas and citrus were retrieved for step 1. Thus no additional search was performed.

3.2.2. Source 1: Agricola

The search strategy translated into this database was:

(survival? OR survive? OR viability OR viable OR mortality)[in Keyword Anywhere] AND (Xanthomonas OR canker? OR cancr?)[in Keyword Anywhere] AND (cit?)[in Keyword Anywhere] AND (treatment? OR management OR operation? OR activit? OR system? OR equipment?)[in Keyword Anywhere]

It retrieved 13 records.

3.2.3. Source 1: CAB Abstracts

Using controlled term (Descriptor) leads to 3 records:

Verdier, E., Zefferino, E., Mendez, S. 2008. Survival of *Xanthomonas citri* subsp. *citri* on the surface of citrus fruit after post harvest treatment Fitopatologia 43(1), 24-31

Vojnov, A. A., Amaral, A. M. do, Dow, J. M., et al. 2010. Bacteria causing important diseases of citrus utilise distinct modes of pathogenesis to attack a common host. Applied Microbiology and Biotechnology 87(2), 467-477

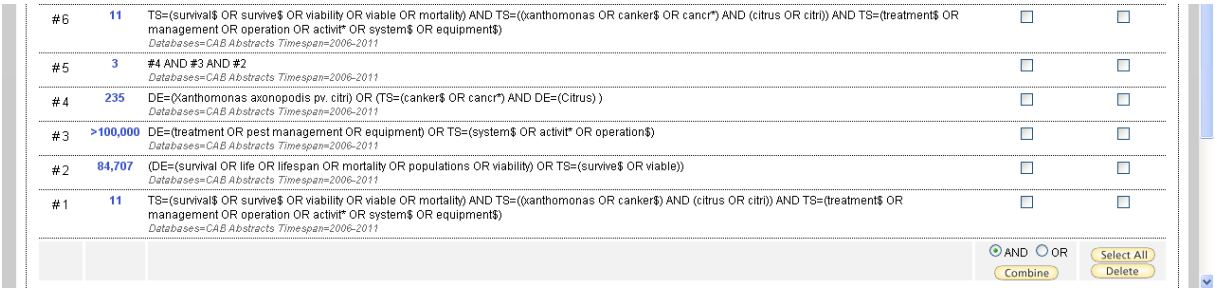
Gottwald, T., Graham, J., Bock, C., et al. 2009. The epidemiological significance of post-packinghouse survival of *Xanthomonas citri* subsp. *citri* for dissemination of Asiatic citrus canker via infected fruit. Crop Protection 28(6), 508-524

A strategy without descriptors retrieved 11 records (including those above) was preferred:

Topic=(survival$ OR survive$ OR viability OR viable OR mortality)
AND Topic=((xanthomonas OR canker$) AND (citrus OR citri))
AND Topic=(treatment$ OR management OR operation OR activit* OR system$ OR equipment$)

Note: "cancr*" retrieved no additional record.

Figure 3: Screenshot of the search realised in CAB Abstracts for step 2

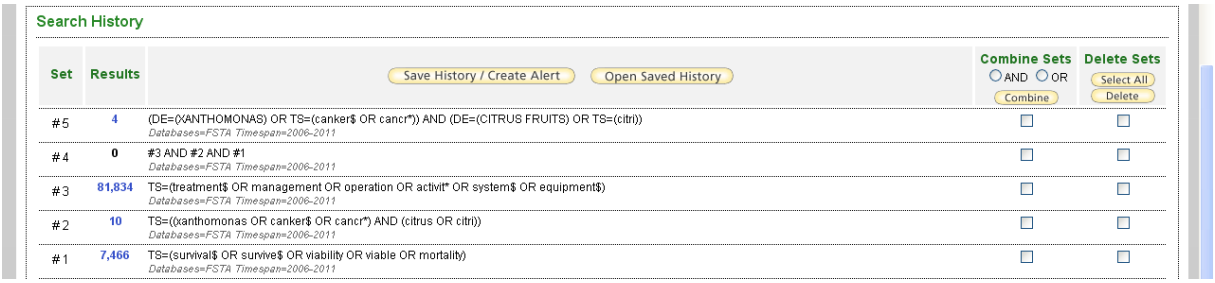


3.2.4. Source 2: FSTA

No controlled term (Descriptor) was used because their use reduced the number of records retrieved (**Error! Reference source not found.**). The combination of concepts used in the other databases retrieved no record. The broader search for the "population" (Xanthomonas and citrus) retrieving a reasonable number of records (10) was preferred.

Topic=((xanthomonas OR canker$ OR cancr*) AND (citrus OR citri))

Figure 4: Screenshot of the search realised in FSTA for step 2



Search History

Set	Results	Save History / Create Alert / Open Saved History	Combine Sets (AND / OR / Combine)	Delete Sets (Select All / Delete)
#5	4	(DE=(XANTHOMONAS) OR TS=(canker$ OR cancr*)) AND (DE=(CITRUS FRUITS) OR TS=(citri)) *Databases=FSTA Timespan=2006-2011*	☐	☐
#4	0	#3 AND #2 AND #1 *Databases=FSTA Timespan=2006-2011*	☐	☐
#3	81,834	TS=(treatment$ OR management OR operation OR activit* OR system$ OR equipment$) *Databases=FSTA Timespan=2006-2011*	☐	☐
#2	10	TS=((xanthomonas OR canker$ OR cancr*) AND (citrus OR citri)) *Databases=FSTA Timespan=2006-2011*	☐	☐
#1	7,466	TS=(survival$ OR survive$ OR viability OR viable OR mortality) *Databases=FSTA Timespan=2006-2011*	☐	☐

3.2.5. Source 3: MedLine

The combination of concepts used in the other databases retrieved 1 record:

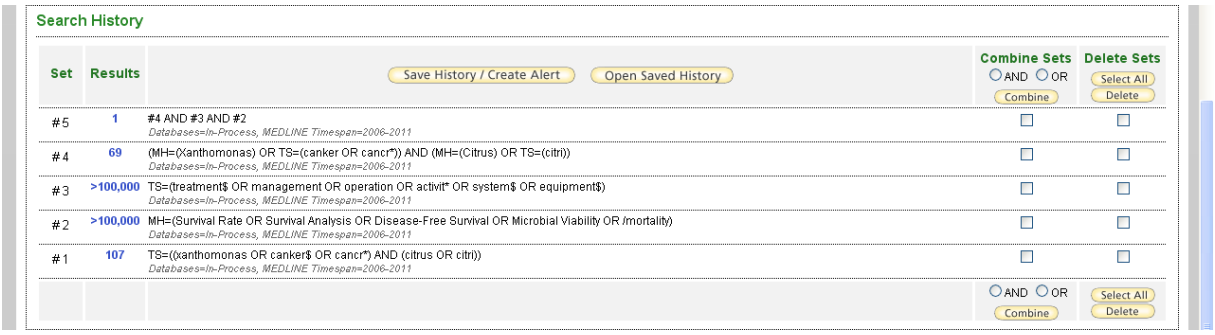
del Campo, Raquel, Russi, Paola, Mara, Pamela, et al. 2009. Xanthomonas axonopodis pv. citri enters the VBNC state after copper treatment and retains its virulence. FEMS Microbiol Lett 298 (2), 143-8.

The broader search for the "population" (Xanthomonas and citrus) retrieving a reasonable number of records (69) was preferred.

Controlled terms are preceded by "MH=".

(MH=(Xanthomonas) OR TS=(canker OR cancr*)) AND (MH=(Citrus) OR TS=(citri))

Figure 5: Screenshot of the search realised in MedLine for step 2



Search History

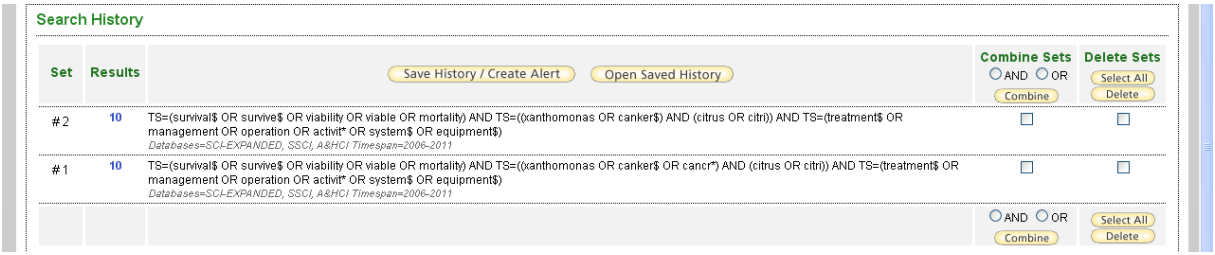
Set	Results	Save History / Create Alert / Open Saved History	Combine Sets (AND / OR / Combine)	Delete Sets (Select All / Delete)
#5	1	#4 AND #3 AND #2 *Databases=In-Process, MEDLINE Timespan=2006-2011*	☐	☐
#4	69	(MH=(Xanthomonas) OR TS=(canker OR cancr*)) AND (MH=(Citrus) OR TS=(citri)) *Databases=In-Process, MEDLINE Timespan=2006-2011*	☐	☐
#3	>100,000	TS=(treatment$ OR management OR operation OR activit* OR system$ OR equipment$) *Databases=In-Process, MEDLINE Timespan=2006-2011*	☐	☐
#2	>100,000	MH=(Survival Rate OR Survival Analysis OR Disease-Free Survival OR Microbial Viability OR /mortality) *Databases=In-Process, MEDLINE Timespan=2006-2011*	☐	☐
#1	107	TS=((xanthomonas OR canker$ OR cancr*) AND (citrus OR citri)) *Databases=In-Process, MEDLINE Timespan=2006-2011*	☐	☐
			AND / OR / Combine	Select All / Delete

3.2.6. Source 4: Web of Science

No controlled terms available.

Topic=(survival$ OR survive$ OR viability OR viable OR mortality)
AND Topic=((xanthomonas OR canker$) AND (citrus OR citri))
AND Topic=(treatment$ OR management OR operation OR activit* OR system$ OR equipment$)

Figure 6: Screenshot of the search realised in Web of Science for step 2



Note: using "cancr*" retrieved no additional record (**Error! Reference source not found.**).

3.3. Step 3: inoculum associated with fruit survives shipment

3.3.1. Source 1: Agris

Records concerning Xanthomonas and citrus were retrieved for step 1. Thus no additional search was performed.

3.3.2. Source 1: Agricola

The translation of the search strategy into that database was:

(survival? OR survive? OR viability OR viable OR mortality)[in Keyword Anywhere] AND (Xanthomonas OR canker? OR cancr?)[in Keyword Anywhere] AND (cit?)[in Keyword Anywhere] AND (shipment? OR shipping? OR transport?)[in Keyword Anywhere]

It retrieved no record.

3.3.3. Source 1: CAB Abstracts

The "survival" concept was relaxed (**Error! Reference source not found.**):

TS=((shipment$ OR shipping$ OR transport$) AND (survival$ OR survive$ OR viability OR viable OR mortality)) AND (DE=(Xanthomonas axonopodis pv. citri) OR (TS=(canker$ OR cancr*)) AND DE=(Citrus))

NOT TS=(nuclear transport OR electron transport OR auxin transport OR ABC-type transport OR ATP-dependent transport)

The search strategy retrieved 3 records:

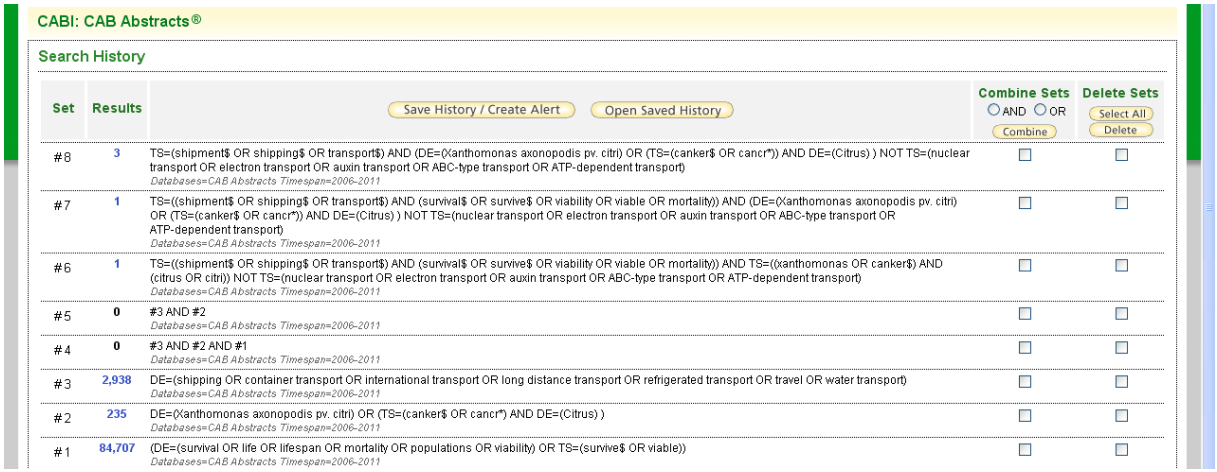
Balasundaram, D., Burks, T.F., Bulanon, D.M., Schubert, T., Lee, W.S., 2009. Spectral reflectance characteristics of citrus canker and other peel conditions of grapefruit. Postharvest Biology and Technology 51, 220-226.

Rayment, G.E., 2006. Australian efforts to prevent the accidental movement of pests and diseases in soil and plant samples, Soil, plant and water analysis: quality analytical tools for an era of ecological

awareness. Papers presented at the 9th International Symposium on Soil and Plant Analysis held in Cancun, Mexico, 30 January-4 February 2005., Taylor & Francis, pp. 2107-2117.

Verdier, E., Zefferino, E., Mendez, S., 2008. Survival of *Xanthomonas citri* subsp. *citri* on the surface of citrus fruit after post harvest treatment. Fitopatologia 43, 24-31.

Figure 7: Screenshot of the search realised in CAB Abstracts for step 3



CABI: CAB Abstracts®

Search History

Set	Results	Save History / Create Alert	Open Saved History	Combine Sets (AND OR Combine)	Delete Sets (Select All Delete)
#8	3	TS=(shipment$ OR shipping$ OR transport$) AND (DE=(Xanthomonas axonopodis pv. citri) OR (TS=(canker$ OR cancr*)) AND DE=(Citrus)) NOT TS=(nuclear transport OR electron transport OR auxin transport OR ABC-type transport OR ATP-dependent transport) *Databases=CAB Abstracts Timespan=2006-2011*		☐	☐
#7	1	TS=((shipment$ OR shipping$ OR transport$) AND (survival$ OR survive$ OR viability OR viable OR mortality)) AND (DE=(Xanthomonas axonopodis pv. citri) OR (TS=(canker$ OR cancr*)) AND DE=(Citrus)) NOT TS=(nuclear transport OR electron transport OR auxin transport OR ABC-type transport OR ATP-dependent transport) *Databases=CAB Abstracts Timespan=2006-2011*		☐	☐
#6	1	TS=((shipment$ OR shipping$ OR transport$) AND (survival$ OR survive$ OR viability OR viable OR mortality)) AND TS=((xanthomonas OR canker$) AND (citrus OR citri)) NOT TS=(nuclear transport OR electron transport OR auxin transport OR ABC-type transport OR ATP-dependent transport) *Databases=CAB Abstracts Timespan=2006-2011*		☐	☐
#5	0	#3 AND #2 *Databases=CAB Abstracts Timespan=2006-2011*		☐	☐
#4	0	#3 AND #2 AND #1 *Databases=CAB Abstracts Timespan=2006-2011*		☐	☐
#3	2,938	DE=(shipping OR container transport OR international transport OR long distance transport OR refrigerated transport OR travel OR water transport) *Databases=CAB Abstracts Timespan=2006-2011*		☐	☐
#2	235	DE=(Xanthomonas axonopodis pv. citri) OR (TS=(canker$ OR cancr*) AND DE=(Citrus)) *Databases=CAB Abstracts Timespan=2006-2011*		☐	☐
#1	84,707	(DE=(survival OR life OR lifespan OR mortality OR populations OR viability) OR TS=(survive$ OR viable)) *Databases=CAB Abstracts Timespan=2006-2011*		☐	☐

3.3.4. Source 2: FSTA

Records concerning Xanthomonas and citrus were retrieved for step 2. Thus no additional search was performed.

3.3.5. Source 3: Medline

Records concerning Xanthomonas and citrus were retrieved for step 2. Thus no additional search was performed.

3.3.6. Source 4: Web of Science

The "survival" concept was relaxed (**Error! Reference source not found.**):

TS=((xanthomonas OR canker$) AND (citrus OR citri)) AND TS=(shipment$ OR shipping$ OR transport$) NOT TS=(nuclear transport OR electron transport OR auxin transport OR ABC* transport* OR ATP-dependent transport)

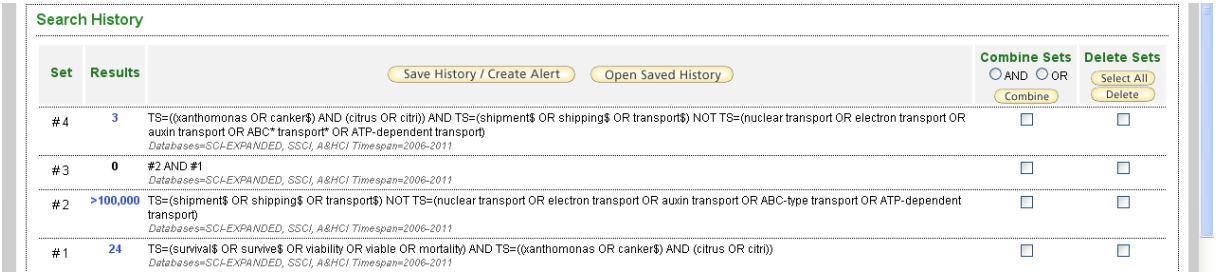
This strategy retrieved 3 records:

Alvarez, L.A., Gramaje, D., Abad-Campos, P., Garcia-Jimenez, J., 2009. Role of the *Helix aspersa* snail as a vector of *Phytophthora citrophthora* causing branch cankers on clementine trees in Spain. Plant Pathology, 58, 956-963.

Balasundaram, D., Burks, T.F., Bulanon, D.M., Schubert, T., Lee, W.S., 2009. Spectral reflectance characteristics of citrus canker and other peel conditions of grapefruit. Postharvest Biology and Technology 51, 220-226.

Souza, L.C.A., Wulff, N.A., Gaurivaud, P., Mariano, A.G., Virgilio, A.C.D., Azevedo, J.L., Monteiro, P.B., 2006. Disruption of *Xylella fastidiosa* CVC gumB and gumF genes affects biofilm formation without a detectable influence on exopolysaccharide production. FEMS Microbiology Letters, 257, 236-242.

Figure 8: Screenshot of the search realised in Web of Science for step 3



4. Results and description of the resulting database

The resulting database contained 413 records. 301 concerned the steps 1, 4 and 5. Eleven of them concerned also the step 2. Five of them concerned also the step 3.

4.1. Number of records found per database for the steps 1, 4 and 5

Database	Records potentially relevant concerning the step 2	General search on citrus canker
Agris	-	+28
Agricola	127	-
CAB Abstracts	199	-
FSTA	-	+10
Medline	-	+69
WoS	190	-

4.2. Number of records found per database for the step 2

Database	Records potentially relevant concerning the step 2	General search on citrus canker
Agris	-	+28
Agricola	13	
CAB Abstracts	11	-

FSTA	0	+10
Medline	1	+69
WoS	11	-

4.3. Number of records found per database for the step 3

Database	Records potentially relevant concerning the step 3	General search on citrus canker
Agris	-	+28
Agricola	0	-
CAB Abstracts	3	-
FSTA	-	+10
Medline	-	+69
WoS	3	-

SCREENING THE RESULTING DATABASE/RECORDS FOR RELEVANCE

The 413 records were equally distributed among five reviewers (scientists, experts of the field) who assessed independently the whole set of evidence for relevance. If the title and the abstract – and, if necessary, the full-text - were judged to be potentially relevant, the record was included. Records were included in the review if the study concerned infection of citrus by *Xanthomonas citri* and their role as source of inoculum. That step was carried out using a standardised form (Distiller SR®). In this form, experts were also asked to attribute the record to a step. Disagreements were resolved by consensus.
All records included at the first relevance screening step, were then again screened for the same purpose, using a new form in Distiller SR®.

B. NOTIFICATIONS OF NON-COMPLIANCE

EPPO Secretariat publishes in the EPPO Reporting Service reports on notifications of non-compliance made because of detection of pests. The EPPO Secretariat points out that the reports are only partial, as many countries have not yet sent their notifications. An overview about the notifications of non-compliance made because of detection of *Xanthomonas citri* subsp. *citri* from 2000 and published in the EPPO Reporting Service is given in the table 1.

Table 1: Interceptions of *Xanthomonas citri* subsp. c*itri* (*Xanthomonas axonopodis* pv. *citri* – *Xac*) notified in the EU from year 2000. Data source: EPPO Reporting Service, 2000 - 2011

Year	Pest	Host	Host intercepted	Origin	Region in which found	Number of notifications
2000	Xac	*Citrus hystix*	Fruits	Thailand	France	1
2001	Xac	*Citrus reticulata*	Fruits	Argentina	Netherlands	4
2002	*Xanthomonas axonopodis*	*Citrus limon*	Fruits	Argentina	France	1
2003	Xac	*Citrus maxima*	Fruits	Thailand	France	1
2004	Xac	*Citrus latifolia*	Fruits	Mexico	Spain	1
2004	*Xanthomonas*	*Citrus clementina*	Fruits	Argentina	Spain	3
2005	Xac	*Citrus sinensis*	Fruits	Uruquay	Spain	10
2006	Xac	*Citrus*	Fruits	Bangladesh	United Kingdom	2
2006	Xac	*Citrus aurantiifolia*	Fruits	Bangladesh	United Kingdom	6
2006	Xac	*Citrus limon*	Fruits	India	United Kingdom	1
2006	*Xanthomonas* (suspect Xac)	*Citrus*	Fruits	Bangladesh	United Kingdom	1
2006	*Xanthomonas*	*Citrus*	Fruits	Bangladesh	United Kingdom	1
2007	Xac	*Citrus*	Fruits	Bangladesh	United Kingdom	7
2007	Xac	*Citrus*	Fruits	India	United Kingdom	3
2007	Xac	*Citrus*	Fruits	Pakistan	United Kingdom	1
2007	Xac	*Citrus aurantiifolia*	Fruits	Bangladesh	United Kingdom	12
2007	Xac	*Citrus aurantiifolia*	Fruits	India	United Kingdom	3
2007	Xac	*Citrus limon*	Fruits	Bangladesh	United Kingdom	1
2007	Xac	*Citrus limon*	Fruits	India	United Kingdom	2
2007	Xac	*Citrus limon*	Fruits	Uruquay	Greece	1
2007	Xac	*Citrus*	Fruits	Thailand	United Kingdom	1
2008	Xac	*Citrus*	Fruits	Bangladesh	United Kingdom	2
2008	Xac	*Citrus*	Fruits	Pakistan	United Kingdom	1
2008	Xac	*Citrus*	Fruits	India	United	1

					Kingdom	
2008	Xac	*Citrus aurantiifolia*	Fruits	Bangladesh	United Kingdom	6
2008	Xac	*Citrus aurantiifolia*	Fruits	India	United Kingdom	5
2008	Xac	*Citrus latifolia*	Fruits	Pakistan	United Kingdom	1
2008	Xac	*Citrus limettioides*	Fruits	Pakistan	United Kingdom	1
2008	Xac	*Citrus limon*	Fruits	India	United Kingdom	1
2008	*Xanthomonas*	*Citrus aurantiifolia*	Fruits	Bangladesh	United Kingdom	3
2008	*Xanthomonas*	*Citrus limon*	Fruits	Bangladesh	United Kingdom	1
2009	Xac	*Citrus*	Fruits	Bangladesh	United Kingdom	7
2009	Xac	*Citrus*	Fruits	India	United Kingdom	2
2009	Xac	*Citrus aurantiifolia*	Fruits	Bangladesh	United Kingdom	7
2009	Xac	*Citrus aurantiifolia*	Fruits	India	United Kingdom	2
2009	Xac	*Citrus aurantiifolia*	Fruits	Pakistan	United Kingdom	1
2009	Xac	*Citrus aurantiifola*	Fruits	Pakistan	United Kingdom	1
2009	Xac	*Citrus limon*	Fruits	Argentina	France	1
2009	Xac	*Citrus limon*	Fruits	India	United Kingdom	1
2009	Xac	*Citrus sinensis*	Fruits	Argentina	Spain	2
2010	Xac	*Citrus*	Fruits	Bangladesh	United Kingdom	14
2010	Xac	*Citrus aurantiifolia*	Fruits	Bangladesh	United Kingdom	11
2010	Xac	*Citrus latifolia*	Fruits	Bangladesh	United Kingdom	2
2010	Xac	*Citrus sinensis*	Fruits	Uruguay	Greece	1
2011	Xac	*Citrus aurantiifolia*	Fruits	Bangladesh	United Kingdom	1

C. EVALUATION OF DIFFERENT EXPERIMENTAL SETTINGS ON CITRUS CANKER - STATISTICAL ISSUES

TABLE OF CONTENTS

BACKGROUND

This technical report has been written by EFSA Scientific Assessment Support Unit to support the PLH scientific opinion for the following request:

> "Request from the USA regarding export of Florida citrus fruits to the EU" (EFSA-Q-2010-01262)"

The following documents are provided by the PLH unit and used in this report:

- **Gottwald et al., 2009.** Gottwald T, Graham J, Bock C, Bonn G, Civerolo E, Irey M, Leite R, McCollum G, Parker P, Ramallo J, Riley T, Schubert T, Stein B, Taylor E: The epidemiological significance of post-packinghouse survival of *Xanthomonas citri* subsp. *citri* for dissemination of Asiatic citrus canker via infected fruit. Crop protection 28, 508-524.

- **Shiotani et al., 2009.** Shiotani H, Uematsu H, Tsukamoto T, Shimizu Y, Ueda K, Mizuno A, Sato S: Survival and dispersal of *Xanthomonas citri* pv. *citri* from infected Satsuma mandarin fruit. Crop protection 28, 19-23.

- **Ploper et al. (2004).** Ploper LD, Ramallo C, Fogliata GM: Proposal for monitoring citrus farms according to packing plants ability to remove fruits with quarantine diseases symptoms. Technical Report, 2004. Annex VIII to IPPC Report of the Second Meeting of the Expert Working Group on "The Use of Integrated Measures in a Systems Approach for Pest Risk Management". Internet: www.ippc.net, last access on 28/03.2011
  .

OBJECTIVES AND SCOPE OF THE REVIEW

The objective of this document is to:

- Evaluate the statistical issues of the references

It is important to note that the scope of this review is limited here to the statistical issues.

1. 1st experiment: Fruit Sampling (Shiotani et al., 2009)

1.1. Screening of the documentation / description of datasets

Source:
Shiotani et al., 2009. Shiotani H, Uematsu H, Tsukamoto T, Shimizu Y, Ueda K, Mizuno A, Sato S: Survival and dispersal of *Xanthomonas citri* pv. *citri* from infected Satsuma mandarin fruit. Crop Protection 28, 19-23.

1.1.1. Description of the proposed risk reduction option

Item	*Description based on the submitted document(s)*	*Comments*
Description of the proposed risk reduction option		
Target pest	Citrus Canker caused by bacterial pathogen *Xanthomonas citri pv. citri*	

	(Hasse)	
Target plant material/product	Mature Satsuma mandarins, *Citrus unshi* Marc.	
Origin of plant material/product	Comercial orchards in Saga, Japan	
Type of risk reduction option	Mature Satsuma mandarin fruits are not carrying the pathogen / The results suggest that there are low numbers of bacteria within lesions on mature fruit of Satsuma mandarin. The bacteria appear to be short-lived after fruits are detached from the tree.	
Place of implementation	Japan	
Other relevant information		

1.1.2. Experimental assessment of the option efficacy to reduce pest infestation in plant material/product under operational conditions

Source:
Shiotani et al., 2009. Shiotani H, Uematsu H, Tsukamoto T, Shimizu Y, Ueda K, Mizuno A, Sato S: Survival and dispersal of *Xanthomonas citri* pv. *citri* from infected Satsuma mandarin fruit. Crop Protection 28, 19-23.

Item	***Description based on the submitted document(s)***	***Comments***
Experimental assessment of the option efficacy to reduce pest infestation in plant material/product <u>under operational conditions</u>		
Plant material information		
Type of plant material/product used in the experiment	Mature Satsuma mandarins from diseased trees / trees severely infected with citrus canker Mandarins with and without symptoms	
Plant identity (e.g. botanical name, variety)	*Citrus unshi Marc.*	
Conditions under which plant materials/products are managed	Commercial orchards in Saga city, Japan / harvested in December 2005 and 2006	
Conditions of the plant commodity (e.g. degree of ripeness, presence of bark, etc.)	Stored at 5 °C / fruits maintained their colour and firmness throughout the study period. No pest control (spraying, further handling or treatment) of trees until assays were conducted	
Pest information		
Identity (species- strains biotypes if applicable-)	Different detection methods (bioassay on Naval oranges, *Citrus sinensis* Osbeck, PCR) without discussion of detection limits	
Conditions under which the pests are cultured, reared or grown	Natural conditions / severely infected trees	
Method of infestation	Natural	

Level of infestation Stage of the pest that is most resistant to the treatment Was the most resistant stage used in the experiment? Potential development of resistance to the option	Severely infested trees / disease index for fruits based on number of lesions (disease severity)	
Experiment(s) description and analysis Variables used to measure efficacy Factors influencing efficacy which were taken into account in the experiment Factors influencing efficacy which were **not** taken into account in the experiment Description of facilities and equipment Description of treatment Monitoring of critical parameters Description of experimental design Presentation of the data Description of the statistical analysis Conclusions of the experiment Other relevant information	 Number of harvested fruits with or without symptoms Disease severity index / fruits with or without symptoms Climatic conditions Description of extraction, bioassay and PCR equipment No treatment Calculation of the average disease index Mandarins with or without symptom in two years Mean severity index, incidence of symptoms and number of mandarins with detected bacteria per year / None None of the templates prepared from harvested fruits yields positive PCR results / The bio assay also gave a negative result; the inoculum prepared from fruits failed to produce canker symptoms in navel oranges	

1.1.3. Extracted data

Shiotani et al. (2009) define the disease index as weighted average of the relative occurrence of 5 classes (0 to 4).

Table 1: Definition of the disease severity index

class	*index*	*no lesions*	*weight*	**rel. occurrence [%] =100 * n_i / n_+**
0	0	0	0/7 = 0	not given
1	1	1-3	1/7 = 0.14	not given
2	3	4-10	3/7 = 0.43	not given

3	5	11-20	5/7 = 0.71	not given
4	7	21 and more	7/7 = 1	not given

Table 2: Detected Citrus Canker on mandarins from infected trees (Shiotani et al. 2009, table 1)

Year	Disease severity average	Fruits asymptomatic	symptomatic	total	Symptoms Incidence rate	Citrus Canker No. of detections	Infection rate	CI	CI
2005	7.5	2208	733	2941	24.9%	0	0.00%	0.00%	0.10%
2006	18	1283	728	2011	36.2%	0	0.00%	0.00%	0.15%

1.2. Data analysis / methods

To express the uncertainty we calculated the 95% confidence intervals for the estimator of the infection rate for all and only symptomatic fruits. All confidence intervals were calculated using Pearson-Clopper intervals, approximated by a F-distribution. (Newcombe 1998, Hartung 2002)

Table 3: Detected Citrus Canker on mandarins from infected trees (Shiotani et al. 2009, table 1)

Year	*Disease severity* average	*Fruits* asymptomatic	symptomatic	total	*Symptoms* Incidence rate	*Citrus Canker* No. of detections	Infection rate	CI	CI
2005	7.5	2208	733	2941	24.9%	0	0.00%	0.00 %	0.10 %
2006	18	1283	728	2011	36.2%	0	0.00%	0.00 %	0.15 %
2005	30	not used	733	733	100%	0	0.00%	0.00 %	0.41 %
2006	50	not used	728	728	100%	0	0.00%	0.00 %	0.41 %

1.3. Results / uncertainties

- Because of lacking information on the sampling scheme the estimated incidence rates provide no information on infection levels in Japan / or if these values are typical
- The severity index is very artificial and gives no real information on the existing severity of the infection. The distribution of the observations on the different classes is missing. The average number of lesions is not calculated.
- The total sample size is high, but no stratified information on the severity classes is given.

- When the sample is representative for the export, then more than 99.85% of all fruits are free from bacteria.
- The detection methods (bioassay, PCR) may not be appropriate.

2. 2nd (a) experiment: Potential Spread (Shiotani et al., 2009)

2.1. Screening of the documentation / description of datasets

Source:
Shiotani et al., 2009. Shiotani H, Uematsu H, Tsukamoto T, Shimizu Y, Ueda K, Mizuno A, Sato S: Survival and dispersal of *Xanthomonas citri* pv. *citri* from infected Satsuma mandarin fruit. Crop Protection 28, 19-23.

2.1.1. Description of the proposed risk reduction option

Item	*Description based on the submitted document(s)*	*Comments*
Description of the proposed risk reduction option		
Target pest	Citrus Canker caused by bacterial pathogen *Xanthomonas citri pv. citri* (Hasse)	
Target plant material/product	Mature Satsuma mandarins, *Citrus unshi* Marc.	
Origin of plant material/product	Commercial orchard in Uki city, Kumamoto Prefecture, Japan	
Type of risk reduction option	Bacterial spread is not possible by normal rainfall	
Place of implementation	Japan	
Other relevant information		

2.1.2. Experimental assessment of the option efficacy to reduce pest infestation in plant material/product under laboratory/controlled conditions

Source:
Shiotani et al., 2009. Shiotani H, Uematsu H, Tsukamoto T, Shimizu Y, Ueda K, Mizuno A, Sato S: Survival and dispersal of *Xanthomonas citri* pv. *citri* from infected Satsuma mandarin fruit. Crop protection 28, 19-23.

Item	*Description based on the submitted document(s)*	*Comments*
Experimental assessment of the option efficacy to reduce pest infestation in plant material/product under laboratory/controlled conditions		
Plant material information		
Type of plant material/product	Naval oranges as bio assay	

used in the experiment Plant identity (e.g. botanical name, variety) Conditions under which plant materials/products are managed Conditions of the plant commodity (e.g. degree of ripeness, presence of bark, etc.)	 *Citrus sinensis* Osbeck Commercial orchard	
Pest information Identity (species- strains biotypes if applicable-) Conditions under which the pests are cultured, reared or grown Method of infestation Level of infestation Stage of the pest that is most resistant to the treatment Was the most resistant stage used in the experiment? Potential development of resistance to the option Experiment(s) description and analysis Variables used to measure efficacy Factors influencing efficacy which were taken into account in the experiment Factors influencing efficacy which were not taken into account in the experiment Description of facilities and equipment Description of treatment Monitoring of critical parameters Description of experimental design	 *X. citri* pv. *citri*, marked strain (KC21Rif100) Resistant to rifampicin, shown to be pathogenic as other strains of *X. citri* pv. *citri* in infection studies. Mature Satsuma mandarins were soaked in a $1x10^6$ cfu per ml bacterial suspension of marked strain KC21Rif100 for 5 minutes. / Concentration approximates the highest levels of bacteria exuded von lesions on leaves of *Citrus natsudaidai* into rainwater. / Kept in dry conditions, room temperature for 24h Unknown 100 trees prepared / in Nov. 2005, Mar. 2006, May. 2006 Detection of *X. citri* pv. *citri* in two (Nov. 05, Mar. 05) resp. four (Oct. 2006) rain traps below each bag in the trees / collection of water after each rainfall / visual inspection of leaves beneath the bags to observe citrus canker disease, detection on lesions Amount of rainfall, dilutions by previous rainfalls 2 contaminated / infected fruits were packed into a polypropylene net bag / two bags were hung in the middle of a naval orange tree Measurement of rainfall Test of Xcc positive in water or lesions beneath the bags with contaminated fruits	In Oct. 2006 four traps below each bag / information on May 06 is missing

Presentation of the data	Data on water samples presented for two days (rainfalls) in Nov. 2005, 3 days in March 2006 and one day in October 2006, but no data for May 2006. Data on diseased leaves (no. of lesions, Xcc positive) for all experiments, assessed 5 month later (Nov. 2005), 4-6 weeks later (March 2006, May 2006) and 2 month later (Oct. 2006)	
Description of the statistical analysis	None	
Conclusions of the experiment	The wild strain of *X. citri* pv. *citri* was detected in rainwater trapped beneath Satsuma mandarin fruits discarded in the orchard. Thus the rain traps used caught the dispersing bacterial cells. However, strain KC21Rif100 was not detected. / Citrus Canker infection caused by the wild strain indicated that the conditions were conducive, however the strain responsible for these lesions was not strain KC21Rif100.	
Other relevant information		

2.1.3. Extracted data

Table 4: Results of spread experiment of contaminated fruits to rain water

Experiment	*Sampling date*	*Rainfall*	*No trees*	*No. bags / tree*	*No. traps / bag*	*No. traps*	*Examinated traps*		*Xcc positive*	*Inspection date*	*No. diseased leave*	*No. lesions*	*No. lesions with Xcc*
		[mm]					abs	rel	abs		abs		abs
Nov. 2005	07th Nov. 05	45	100	2	2	400	170	43%	0				
	12th Nov. 05	5	100	2	2	400	85	21%	0	06th Mar. 06	0		
Mar. 2006	10th Mar. 06	4	100	2	2	400	176	44%	0				
	17th Mar. 06	12	100	2	2	400	214	54%	0				
	23rd Mar. 06	15	100	2	2	400	227	57%	0	08th May.06	0		
May 2006		unknown	100	2	2	400	unknown			12th Jun. 06	38	113	0
Oct. 2006	23rd Oct. 06	13	100	2	4	800	32	4%	0	20th Nov. 06	0		

2.2. Data analysis / methods

Table 5: Results including 95% confidence intervals for Xcc positive testing in rain water or lesions of the spread experiment

Experiment	*Sampling date*	*Rainfall*	*No trees*	*No. traps*	*Examinated traps*	*Xcc positive*				*Inspection date*	*No. diseased leave*	*No. lesions*	*No. lesions with Xcc*			
		[mm]			abs	abs	rel	CI	CI		abs		abs	rel	CI	CI
Nov. 2005	07th Nov. 05	45	100	400	170	0	0%	0%	1.75%							
	12th Nov. 05	5	100	400	85	0	0%	0%	3.46%	06th Mar. 06	0					
Mar. 2006	10th Mar. 06	4	100	400	176	0	0%	0%	1.69%							
	17th Mar. 06	12	100	400	214	0	0%	0%	1.39%							
	23rd Mar. 06	15	100	400	227	0	0%	0%	1.31%	08th May.06	0					
May 2006		miss.	100	400						12th Jun. 06	38	113	0	0%	0%	2.62%
Oct. 2006	23rd Oct. 06	13	100	800	32	0	0%	0%	8.94%	20th Nov. 06	0					

2.3. Results / uncertainties

- The number and selection of examined traps is unclear and small (between 4% and 57% of all traps).
- The detection limit of sampling beneath bags is unknown. The influence of the amount of rain is unclear. What is the dilution effect of rain, don't going through the net bag with infected fruits? What is the effect of multiple rainfalls per experiment?
- The sampling in May 2006 is not described.
- No information is provided on the time between infection / run out and rainfall
- No information is given on start of rotting (in March more than 14 days)

3. 2nd (b) experiment: Survival of bacteria on Satsuma mandarins under orchard conditions (Shiotani et al., 2009)

3.1. Screening of the documentation / description of datasets

Source:
Shiotani et al., 2009. Shiotani H, Uematsu H, Tsukamoto T, Shimizu Y, Ueda K, Mizuno A, Sato S: Survival and dispersal of *Xanthomonas citri* pv. *citri* from infected Satsuma mandarin fruit. Crop Protection 28, 19-23.

3.1.1. Description of the proposed risk reduction option

Item	*Description based on the submitted document(s)*	*Comments*
Description of the proposed risk reduction option		
Target pest	Citrus Canker caused by bacterial pathogen *Xanthomonas citri pv. citri* (Hasse)	
Target plant material/product	Mature Satsuma mandarins, *Citrus unshi* Marc.	
Origin of plant material/product	Commercial orchard in Uki city, Kumamoto Prefecture, Japan	
Type of risk reduction option	Bacterial spread is not possible by normal rainfall	
Place of implementation	Japan	
Other relevant information		

3.1.2. Experimental assessment of the option efficacy to reduce pest infestation in plant material/product under laboratory/controlled conditions

Source:
Shiotani et al., 2009. Shiotani H, Uematsu H, Tsukamoto T, Shimizu Y, Ueda K, Mizuno A, Sato S: Survival and dispersal of *Xanthomonas citri* pv. *citri* from infected Satsuma mandarin fruit. Crop Protection 28, 19-23.

Item	*Description based on the submitted document(s)*	*Comments*
Experimental assessment of the option efficacy to reduce pest infestation in plant material/product under laboratory/controlled conditions		
Plant material information Type of plant material/product used in the experiment Plant identity (e.g. botanical name, variety) Conditions under which plant materials/products are managed Conditions of the plant commodity (e.g. degree of ripeness, presence of bark, etc.)	 Mature Satsuma mandarin fruits Satsume mandarins, *Citrus unshi* Marc. Commercial orchard	 Naval oranges as bio assay *Citrus sinensis* Osbeck
Pest information Identity (species- strains biotypes if applicable-) Conditions under which the pests are cultured, reared or grown Method of infestation Level of infestation Stage of the pest that is most resistant to the treatment Was the most resistant stage used in the experiment? Potential development of resistance to the option Experiment(s) description and analysis Variables used to measure efficacy Factors influencing efficacy which were taken into account in the experiment Factors influencing efficacy which were not taken into account in the experiment Description of facilities and equipment Description of treatment	 *X. citri* pv. *citri*, marked strain (KC21Rif100) Resistant to rifampicin, shown to be pathogenic as other strains of *X. citri* pv. *citri* in infection studies. Mature Satsuma mandarins were soaked in a $1x10^6$cfu per ml bacterial suspension of marked strain KC21Rif100 for 5 minutes. / Concentration approximates the highest levels of bacteria exuded von lesions on leaves of *Citrus natsudaidai* into rainwater. / Kept in dry conditions, room temperature for 24h Unknown 8 Naval orange trees prepared May 2007 with 5 bags containing 4 contaminated mandarin fruits. Detection of *X. citri* pv. *citri* on 20 fruits after 0 / 3 / 6 / 9 / 12 / 15 and 21 days under orchard conditions by bio assay on leaves of Naval oranges Amount and number of rainfalls 4 contaminated / infected fruits were packed into a polypropylene net bag / five bags were hung in the middle of a	

	naval orange tree	
Monitoring of critical parameters		
Description of experimental design	Test of Xcc positive on peel of contaminated fruits	
Presentation of the data	Number of rotted fruits and mean (and standard deviation) number of lesions per fruit for 7 time points	
Description of the statistical analysis	Mean und standard deviation	
Conclusions of the experiment	The inocula prepared from contaminated fruit's rinds retrieved after 3 days in the orchard did not cause any canker symptoms on attached leaves of Naval oranges.	
Other relevant information		

3.1.3. Extracted data

Table 6: Detection of Xcc on Satsuma mandarin under different number of days under orchard conditions

Days under orchard conditions	*Sampled fruits*		*Mean (std dev) number of lesions per fruit*
	Total	Rotted	
0	20	0	1.4 (0.5)
3	20	0	0
6	20	0	0
9	20	2	0
12	20	3	0
15	20	5	0
21	20	11	0

3.2. Data analysis / methods

Table 7: Rate of symptoms (lesions) on contaminated Satsuma mandarin after different days under orchard conditions

Days under orchard conditions	*Sampled fruits*			*Number of fruits with lesions*			
	Total	Rotted		abs	rel	Confidence interval	
		abs	rel				
0	20	0	0%				
3	20	0	0%	0	0%	0%	13.91%
6	20	0	0%	0	0%	0%	13.91%
9	20	2	10%	0	0%	0%	13.91%
12	20	3	15%	0	0%	0%	13.91%

15	20	5	25%	0	0%	0%	13.91%
21	20	11	55%	0	0%	0%	13.91%

3.3. Results / uncertainties

- The sample size is too small to give clear results on the survival on the surface. The upper limit of the confidence interval for existence of lesions is about 14%.
- The detection method might be not sensitive.
- The evaluation of fruits 3 month after inoculation showed one two three lesions per fruit. The chosen maximum duration of 21 days might be too short.

4. Packing inspection, table 2 of Ploper et al. (2004)

4.1. Screening of the documentation / description of datasets

Source:
Ploper et al. (2004). Ploper LD, Ramallo C, Fogliata GM: Proposal for monitoring citrus farms according to packing plants ability to remove fruits with quarantine diseases symptoms. Technical Report, 2004. Annex VIII to IPPC Report of the Second Meeting of the Expert Working Group on "The Use of Integrated Measures in a Systems Approach for Pest Risk Management". Internet: www.ippc.net, last access on 28/03.2011.

4.1.1. Description of the proposed risk reduction option

Item	*Description based on the submitted document(s)*	*Comments*
Description of the proposed risk reduction option		
Target pest	Citrus Canker	
Target plant material/product	Citrus (fresh) fruits	
Origin of plant material/product	Argentina	
Type of risk reduction option		EC 416/2004 demands: no symptom at place of production from beginning of vegetative cycle / asymptomatic harvested fruits / free from bacteria / appropriate treatment of disinfection / thorough record of the chain
Place of implementation	Packing plant in Tucumán / Argentina	
Other relevant information	Fruit destination outside EU	

4.1.2. Experimental assessment of the option efficacy to reduce pest infestation in plant material/product under operational conditions

Source:

Ploper et al. (2004). Ploper LD, Ramallo C, Fogliata GM: Proposal for monitoring citrus farms according to packing plants ability to remove fruits with quarantine diseases symptoms. Technical Report, 2004. Annex VIII to IPPC Report of the Second Meeting of the Expert Working Group on "The Use of Integrated Measures in a Systems Approach for Pest Risk Management". Internet: www.ippc.net, last access on 28/03.2011.

Item	*Description based on the submitted document(s)*	*Comments*
Experimental assessment of the option efficacy to reduce pest infestation in plant material/product under operational conditions		
Plant material information		
Type of plant material/product used in the experiment	Dumped fruits in the weeks 25 to 31 of 2004 (corresponding June, July 2004) in Tucumán, Argentina Total number of packed fruits: 336 360 924 / total number of Citrus Canker: 43 149 (0.1005%), further specified per week. Fruits came from different origins, chosen at random, located at different agroecological areas in Tucumán.	
Plant identity (e.g. botanical name, variety)	Citrus fruits	
Conditions under which plant materials/products are managed	Real conditions of a normal, commercial packing house	
Conditions of the plant commodity (e.g. degree of ripeness, presence of bark, etc.)		
Pest information		
Identity (species- strains biotypes if applicable-)		
Conditions under which the pests are cultured, reared or grown	Natural	
Method of infestation	Natural	
Level of infestation	Average over all weeks is that 0.0128% of all dumped fruits are symptomatic	
Stage of the pest that is most resistant to the treatment		(refer to research data if relevant)
Was the most resistant stage used in the experiment?		
Potential development of resistance to the option		
Experiment(s) description and analysis		
Variables used to measure efficacy	Number of detected, symptomatic fruits in the inspection line. Evaluation	

	was done by number of detected, symptomatic fruits after the inspection line (at bench)	
Factors influencing efficacy which were taken into account in the experiment	None	
Factors influencing efficacy which were **not** taken into account in the experiment	Number of dumped fruits per day / actual infection rate / type of citrus fruits /	
Description of facilities and equipment	0^{th} inspection (sampling) at dump / 1^{st} inspection after washing and soda rinsing / 2^{nd} inspection after disinfection and drying / final inspection after waxing	
Description of treatment	Visual inspection (4 steps) in an inspection line	
Monitoring of critical parameters		
Description of experimental design	The remaining number of symptomatic fruits after the inspection line / estimated by additional visual inspection at bench / Visual detection has a detection threshold of canker of approximately 1 to 2mm. / Quantifications were made by guess	
Presentation of the data	On 8 days/locations: total number of fruits / no. of symptomatic fruits on sampling / 1^{st} inspection / 2^{nd} inspection / inspection line / at bench / no. of packed boxes with symptomatic fruits	
Description of the statistical analysis	Calculation of rates (in relation to the number of dumped fruits)	
Conclusions of the experiment	It shows that dumps entering the packing plant with infection rates of about 1%, they arrive at the bench with values almost reaching 0 and at the box with no symptoms.	
Other relevant information		

4.1.3. Extracted data

Ploper et al. (2004) estimated the average infection rate per week to be 0.01%, with variation 0.002% (week 29) to 0.036% (week 25). The total number of fruits was calculated by the number of trays dumped in the packing house multiplied by 135 fruits per tray.

Table 8: Number of fruits and detected infections per week (in 2004) in Tucumán (Ploper et al. 2004, Chart 1)

Week	*Dumped trays*	*no. fruits*	*fruits/tray*	*Tatal Canker*	*Infection rate (Average weighted)*
25	244202	33699876	138	12262	0.0364%
26	297540	41060520	138	6973	0.0170%
27	341560	47135280	138	7678	0.0163%
28		48842340		4435	0.0091%
29	388870	53664060	138	898	0.0017%
30	425626	58736388	138	2757	0.0047%
31	385670	53222460	138	8146	0.0153%
Total	2083468	336360924		43149	
Average	347245	48051561	138	6164	0.0128%

Ploper et al. (2004) reported for 8 days in period of 10th June to 07th August 2004 the number of fruits at entry and the numbers of detected symptomatic fruits on several stages of the inspection line:

1. Sampling of symptomatic fruits at the dumping (0th inspection)
2. 1st visual inspection after washing and soda rinsing (1st inspection)
3. 2nd visual inspection after drying (2nd inspection)
4. 3rd and final visual inspection after waxing (End of inspection line)

A final control was made after the inspection line (at bench) to evaluate the effectiveness of the inspection line. The inspection of boxes is not described in details.

Table 9: Number of detected symptomatic, and remaining healthy fruits in the inspection line (Ploper et al. 2004, Chart 2)

Date	*Week/WD*	*No. fruits at entry*	*No. sampled fruits*	*No. remaining fruits after 0th inspection*	*No. symptomatic fruits at 1st inspection*	*No. remaining fruits after 1st inspection*	*No. symptomatic fruits at 2nd inspection*	*No. remaining fruits after 2nd inspection*	*No. symptomatic fruits at inspection line*	*No. fruits after inspection line*	*No. symptomatic fruits at bench*	*No. remaining fruits at bench*	*No. of boxes with symptomatic fruits*
A		B	C	D=B-C	E	F=D-E	G	H=F-G	I	J=H-I	K	L=K-J	M
10/06.2004	24/Thu	173880	6694	167186	412	166774	164	166610	19	166591	2	166589	0
30/07.2004	31/Fri	331200	11228	319972	194	319778	78	319700	4	319696	2	319694	0
17/06.2004	25/Thu	162840	2182	160658	76	160582	30	160552	0	160552	0	160552	0
30/06.2004	27/Wed	165600	1623	163977	233	163744	47	163697	6	163691	0	163691	0
07/08.2004	32/Sat	160080	912	159168	15	159153	6	159147	0	159147	0	159147	0
16/06.2004	25/Wed	364320	1894	362426	328	362098	175	361923	8	361915	1	361914	0
16/06.2004	25/Wed	314640	787	313853	177	313676	77	313599	2	313597	0	313597	0
21/06.2004	26/Mon	380880	647	380233	122	380111	55	380056	0	380056	0	380056	0
Total		2053440	25967	2027473	1557	2025916	632	2025284	39	2025245	5	2025240	0

Ploper et al. (2004) calculated the infection rates always in relation to the total number of fruits at entry (Column "B").

Table 10: Number of detected infection rates (in relation to fruits at entry) in the inspection line (Ploper et al. 2004, Chart 2)

Date	*Week/WD*	*Rate at 0th inspection*	*Rate at 1st inspection*	*Rate at 2nd inspection*	*Rate at Inspection line*	*Total detected symptomatic fruits*	*Total detection rate after Inspection*	*CI*	*CI*	*Rate at bench*	*CI*	*CI*	*Upper bound of symptomatic fruits passing inspection line*	*Upper bound of infection rate of fruits passing the inspection line*
A		=C/B	=E/B	=G/B	=I/B	N =C+E+G+I	=N/B	O		=K/J		P	=B*(1-O)*P	=(1-O)*P
10/06.2004	24/Thu	3.85%	0.237%	0.094%	0.011%	7289	4.1920%	4.0983%	4.2872%	0.0012%	0.0001%	0.0043%	7	0.0042%
30/07.2004	31/Fri	3.39%	0.059%	0.024%	0.001%	11504	3.4734%	3.4113%	3.5363%	0.0006%	0.0001%	0.0023%	7	0.0022%
17/06.2004	25/Thu	1.34%	0.047%	0.018%	0.000%	2288	1.4051%	1.3485%	1.4634%	0.0000%	0.0000%	0.0019%	3	0.0018%
30/06.2004	27/Wed	0.98%	0.141%	0.028%	0.004%	1909	1.1528%	1.1019%	1.2054%	0.0000%	0.0000%	0.0018%	3	0.0018%
07/08.2004	32/Sat	0.57%	0.009%	0.004%	0.000%	933	0.5828%	0.5461%	0.6213%	0.0000%	0.0000%	0.0019%	3	0.0019%
16/06.2004	25/Wed	0.52%	0.090%	0.048%	0.002%	2405	0.6601%	0.6341%	0.6870%	0.0003%	0.0000%	0.0015%	6	0.0015%
16/06.2004	25/Wed	0.25%	0.056%	0.024%	0.001%	1043	0.3315%	0.3117%	0.3522%	0.0000%	0.0000%	0.0010%	3	0.0010%
21/06.2004	26/Mon	0.17%	0.032%	0.014%	0.000%	824	0.2163%	0.2018%	0.2316%	0.0000%	0.0000%	0.0008%	3	0.0008%
Total		1.26%	0.076%	0.031%	0.002%	28195	1.3731%	1.3572%	1.3891%	0.0002%	0.0001%	0.0006%	12	0.0006%

4.2. Data analysis / methods

We added at every inspection stage the number of remaining healthy fruits at this level.

Table 11: Number of detected symptomatic, and remaining healthy fruits in the inspection line

Date	*Week/WD*	*No. fruits at entry*	*No. sampled fruits*	*No. remaining fruits after 0th inspection*	*No. symptomatic fruits at 1st inspection*	*No. remaining fruits after 1st inspection*	*No. symptomatic fruits at 2nd inspection*	*No. remaining fruits after 2nd inspection*	*No. symptomatic fruits at inspection line*	*No. fruits after inspection line*	*No. symptomatic fruits at bench*	*No. remaining fruits at bench*	*No. of boxes with symptomatic fruits*
A		B	C	D=B-C	E	F=D-E	G	H=F-G	I	J=H-I	K	L=K-J	M
10/06.2004	24/Thu	173880	6694	167186	412	166774	164	166610	19	166591	2	166589	0
30/07.2004	31/Fri	331200	11228	319972	194	319778	78	319700	4	319696	2	319694	0
17/06.2004	25/Thu	162840	2182	160658	76	160582	30	160552	0	160552	0	160552	0
30/06.2004	27/Wed	165600	1623	163977	233	163744	47	163697	6	163691	0	163691	0
07/08.2004	32/Sat	160080	912	159168	15	159153	6	159147	0	159147	0	159147	0
16/06.2004	25/Wed	364320	1894	362426	328	362098	175	361923	8	361915	1	361914	0
16/06.2004	25/Wed	314640	787	313853	177	313676	77	313599	2	313597	0	313597	0
21/06.2004	26/Mon	380880	647	380233	122	380111	55	380056	0	380056	0	380056	0
Total		**2053440**	**25967**	**2027473**	**1557**	**2025916**	**632**	**2025284**	**39**	**2025245**	**5**	**2025240**	**0**

We estimated the remaining infection rates after inspection using the ratio of detected, symptomatic fruits and the number of inspected fruits at this level. Additionally we calculated the total infection rate which was detected on any stage of the inspection line. This should be comparable to the prevalence of Citrus Canker in the origin of the fruits; that is the province of Tucumán (Agentina).

To express the uncertainty we calculated the 95% confidence intervals for the estimator of prevalence and the estimator of the effectiveness of the inspection line. All confidence interval were calculated using Pearson-Clopper intervals, approximated by a F-distribution. (Newcombe 1998, Hartung 2002).

To conclude on the total effectiveness of the inspection line, we calculated the upper level of symptomatic fruits per day passing the inspection, and the upper level of infection rate passing the inspection line. This was done by assuming the lowest rate detected by the inspection multiplied by the highest rate detected in the final evaluation.

Table 12: Number of detected infection rates (in relation to inspected fruits at the inspection level) in the inspection line

Date	*Week/ WD*	*Rate at 0th inspection*	*Rate at 1st inspection*	*Rate at 2nd inspection*	*Rate at Inspection line*	*Total detected symptomatic fruits*	*Total detection rate after Inspection*	*CI*	*CI*	*Rate at bench*	*CI*	*CI*	*Upper bound of symptomatic fruits passing inspection line*	*Upper bound of infection rate of fruits passing the inspection line*
A		=C/B	=E/D	=G/F	=I/H	N =C+E+G+I	=N/B	O		=K/J		P	=B*(1-O)*P	=(1-O)*P
10/06.2004	24/Thu	3.85%	0.246%	0.098%	0.011%	7289	4.1920%	4.0983%	4.2872%	0.0012%	0.0001%	0.0043%	7	0.0042%
30/07.2004	31/Fri	3.39%	0.061%	0.024%	0.001%	11504	3.4734%	3.4113%	3.5363%	0.0006%	0.0001%	0.0023%	7	0.0022%
17/06.2004	25/Thu	1.34%	0.047%	0.019%	0.000%	2288	1.4051%	1.3485%	1.4634%	0.0000%	0.0000%	0.0019%	3	0.0018%
30/06.2004	27/Wed	0.98%	0.142%	0.029%	0.004%	1909	1.1528%	1.1019%	1.2054%	0.0000%	0.0000%	0.0018%	3	0.0018%
07/08.2004	32/Sat	0.57%	0.009%	0.004%	0.000%	933	0.5828%	0.5461%	0.6213%	0.0000%	0.0000%	0.0019%	3	0.0019%
16/06.2004	25/Wed	0.52%	0.091%	0.048%	0.002%	2405	0.6601%	0.6341%	0.6870%	0.0003%	0.0000%	0.0015%	6	0.0015%
16/06.2004	25/Wed	0.25%	0.056%	0.025%	0.001%	1043	0.3315%	0.3117%	0.3522%	0.0000%	0.0000%	0.0010%	3	0.0010%
21/06.2004	26/Mon	0.17%	0.032%	0.014%	0.000%	824	0.2163%	0.2018%	0.2316%	0.0000%	0.0000%	0.0008%	3	0.0008%
Total		1.26%	0.077%	0.031%	0.002%	28195	1.3731%	1.3572%	1.3891%	0.0002%	0.0001%	0.0006%	12	0.0006%

4.3. Results / uncertainties

- The weekly average of the rate of symptomatic fruits at the packing line in Tucumán (weeks 25-31 in 2004) is in the relevant period between 0.0017% and 0.0364%. It is unclear how these figures were obtained.

- Regarding the daily data the rate of symptomatic fruits at the packing line in Tucumán (8days in weeks 24 to 32 in 2004) is between 4.19% and 0.22%. It is unclear how these days were chosen. Some days are not corresponding to the weekly averages mentioned before. For 4 of 8 days the infection rate at dump was higher than 1%. All daily infection rates were higher than the weekly averages.

- After the inspection line (at bench) the remaining infection rate was still up to 0.0012% with an upper confidence of up to 0.0043%. This upper level corresponds to up to 7 symptomatic fruits passing the inspection line per day. It is unclear, why these fruits don't enter the boxes.

- All quantifications of numbers of fruits were done by guess.

- It is not mentioned that the detection methods for evaluation was different from the visual inspection applied in the inspection line.

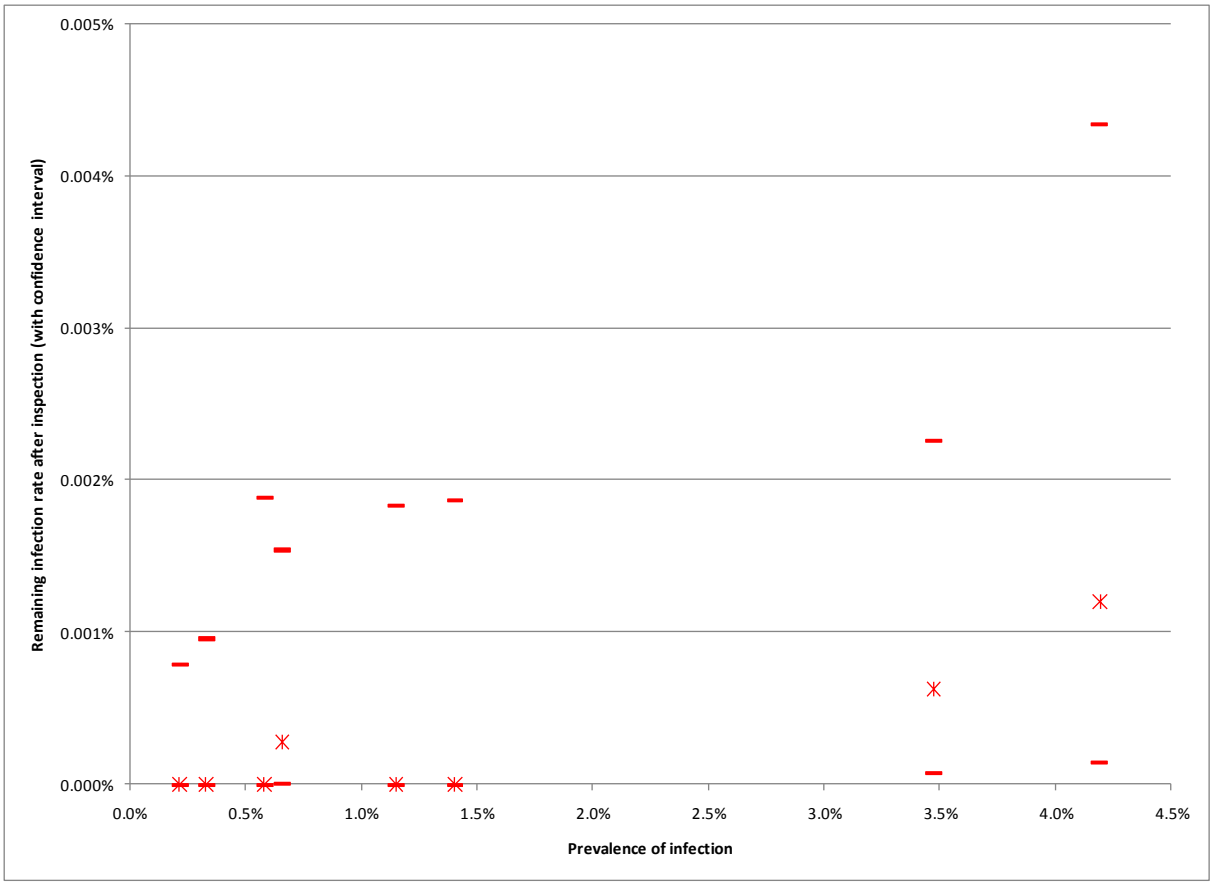


Figure 9: Remaining infection rate in dependence from initial prevalence

- The effectiveness of the inspection line shows a positive trend for increasing prevalence. For prevalence below 1% the upper level of remaining infection rate is still about 0.002%.

5. Prior harvest inspection, tables 3 and 4 of Ploper et al. (2004)

Ploper et al. (2004) propose "an inspection prior to the harvest will be carried out in order to determine the incidence of affected fruit in the production site. The incidence index is determined by the quantity of affected fruit over the total quantity of fruits considered. With an incidence of symptomatic fruit lower or equal 1%, the harvest is authorised to be processed in the packing plants registered for exports".

To confirm that the incidence of symptomatic fruits is lower or equal 1% Ploper et al (2004) calculate the maximal acceptable number of symptomatic fruits per tree by visual inspection. The calculation uses no experimental data and is based only on assumptions.

5.1. Screening of the documentation / description of datasets

Source:
Ploper et al. (2004). Ploper LD, Ramallo C, Fogliata GM: Proposal for monitoring citrus farms according to packing plants ability to remove fruits with quarantine diseases symptoms. Technical Report, 2004. Annex VIII to IPPC Report of the Second Meeting of the Expert Working Group on "The Use of Integrated Measures in a Systems Approach for Pest Risk Management". Internet: www.ippc.net, last access on 28/03.2011.

5.1.1. Description of the proposed risk reduction option

Item	*Description based on the submitted document(s)*	*Comments*
Description of the proposed risk reduction option		
Target pest	Citrus Canker	
Target plant material/product	Citrus trees	
Origin of plant material/product	Argentina	
Type of risk reduction option	Visual inspection of trees and exclusion of trees with incidence of symptomatic fruits higher 1% from harvest.	
Place of implementation	Theoretical model	
Other relevant information		

5.1.2. Assessment of option effectiveness to reduce risk of pest entry from infested area to pest free area

Source:
Ploper et al. (2004). Ploper LD, Ramallo C, Fogliata GM: Proposal for monitoring citrus farms according to packing plants ability to remove fruits with quarantine diseases symptoms. Technical Report, 2004. Annex VIII to IPPC Report of the Second Meeting of the Expert Working Group on "The Use of Integrated Measures in a Systems Approach for Pest Risk Management". Internet: www.ippc.net, last access on 28/03.2011.

Item	*Description based on the submitted document(s)*	*Comments*
Assessment of option effectiveness to reduce risk of pest entry from infested area to pest free area		
Consignments Origin Type of commodities Surveillance method Level of infestation of plant material/product Quantity of commodities Means of transportation	Harvest of one citrus tree Argentina Citrus fruits Visual inspection of all fruits in height from 1.25m to 1.85m Theoretical incidence rates: 1%, 3% and 5% Up to 2, 3 to 4, 5 to 6 and 7 to 8 trays per tree / 135 fruits per tray Transportation to the packing house	
Detection method of the pest in the plant material/product Place(s) of implementation Sampling technique Type of detection method Accuracy	 On the plantation All fruits in height from 1.25m to 1.85m Visual inspection Unknown	
Point(s) of entry	Packing house / no harvest, when rejected	
Variable used to describe probability of pest entry Conclusion of the assessment Other relevant information	Infection rate Depending on the number of inspected fruits, from 1 to 4 symptomatic fruits are acceptable to confirm an infection rate less or equal 1%. Theoretical model	

5.1.3. Extracted data

Table 13: Average number of infected fruits per tree and under visual inspection for trees with different amount of fruits and infection rates. (Ploper et al. 2004, Chart 3 and 4)

				Affected fruits per plant (different infection rates)			*In-spected fruits (30%)*	*Average number of detected symptomatic fruits per plant (different infection rates)*		
Trays /plant	Average	Fruits /tray	Fruits /plant	5.00%	3.00%	1.00%		5.00%	3.00%	1.00%
0-2	1.5	135	203	10.1	6.1	2.0	61	3	2	1
3-4	3.5	135	473	23.6	14.2	4.7	142	7	4	1
5-6	5.5	135	743	37.1	22.3	7.4	223	11	7	2
7-8	7.5	135	1013	50.6	30.4	10.1	304	15	9	3
9-10	9.5	135	1283	64.1	38.5	12.8	385	19	12	4

5.2. Data analysis / methods

Using the Hypergeometric distribution we calculated the probability not to observe (at least one) symptomatic fruit on a tree by visual inspection, when the real infection rate is p_0. E.g. for a tree with 1.5 trays of fruits (203 fruits per tree and of these 61 visual inspected) and an assumed infection rate of 1% (in total 2 symptomatic fruits) the probability is about 50% not to observe at least one symptomatic fruit at visual inspection. Allowing an error probability of α=5% (not reject a tree with infection rate higher p_0=1%) and rejecting a tree when at least one symptomatic fruit was detected, will enable to test only trees with an average of 7.5 or more trays per tree.

Using the normal approximation of the binomial test we calculated the acceptable number of symptomatic fruits per tree at visual inspection. The (nul-)hypothesis of H_0:$p>p_0$ can be rejected (α=5%), when no symptomatic fruits were detected on trees with an average of 7.5 resp. 9.5 trays per tree. The number of symptomatic fruits is too small for trees with an average of 1.5, 3.5 or 5.5 trays per tree to test the hypothesis.

Table 14: Probability of detection and number of acceptable symptomatic fruits to reject a given infection rate for trees with different amount of fruits and infection rates.

		Affected fruits per plant (different infection rates)			*Ins-pected fruits (30%)*	*Probability not to detect (at least one) symptomatic fruit (different infection rates)*			*Accepted no. symptomatic, detected fruits to reject H_0: $p>=p_0$ (different infection rates p_0)*		
Mean trays /plant	Fruits /plant	5.00%	3.00%	1.00%		5%	3%	1%	5%	3%	1 %
1.5	203	10.1	6.1	2.0	61	2.54%	11.34%	48.83 %	0		
3.5	473	23.6	14.2	4.7	142	0.015%	0.62%	16.63 %	2	0	
5.5	743	37.1	22.3	7.4	223	0.00012 %	0.03%	8.12%	5	2	
7.5	1013	50.6	30.4	10.1	304	0.00000 %	0.0019 %	2.77%	8	4	0
9.5	1283	64.1	38.5	12.8	385	0.00000 %	0.0001 %	0.94%	12	6	0

5.3. Results / uncertainties

- The average of symptomatic fruits per inspection cannot be used to confirm a low infection level. This confirmation has to be done by a statistical test.
- The test to confirm that the infection rate is less or equal 1% is only possible for trees with large amount of (inspected) fruits, this means 7.5 or 9.5 trays (1 tray = 20kg) fruits per tree.
- The calculations depend on the number of inspected fruits. It is assumed that 1 tray contains 135 fruits. If this number is smaller (the weight of fruits higher) the number of acceptable fruits will be smaller.

- It is assumed that every fruit in the height between 1.25m and 1.85m will be inspected independently from their location in the tree.
- It is assumed that the infection is equally distributed over the height on a tree.
- It is assumed that 30% of all fruits are in the height between 1.25m and 1.85m. More than 300 fruits have to be inspected per tree. Ploper et al (2004) state that an inspector will be able to efficiently control up to 300 fruits per tree.
- No information on the detection limit of the infection on a single fruit is given.

In summary the confirmation of low infection levels need a large amount of fruits under visual inspections, namely more than 300. These numbers are only possible for trees with large amounts of fruits under harvest, but impractical for inspectors.

REFERENCES

Newcombe R.G. 1998. Two-sided confidence intervals for the single proportion : comparison of seven methods. Statistics in medicine 17, 857-872.

Hartung J, 2002. Statistik- Lehr-und Handbuch, 13. Auflage. München: Oldenbourg, 2002.

Appendix A: ERRO evaluation scheme (Draft Version)

1. Description of the proposed risk reduction option

Item	*Description based on the submitted document(s)*	*Comments*
Description of the proposed risk reduction option		
Target pest **Target plant material/product** **Origin of plant material/product** **Type of risk reduction option** **Place of implementation** **Other relevant information**	(e.g., species, strain) (e.g., species, cultivar) (e.g., heat treatment, fumigation, combination of several treatments)	

1.1. Experimental assessment of the option efficacy to reduce pest infestation in plant material/product under laboratory/controlled conditions

Source (indicate the reference of the supporting documents and data and their confidentiality status if applicable):

Item	*Description based on the submitted document(s)*	*Comments*
Experimental assessment of the option efficacy to reduce pest infestation in plant material/product under laboratory/controlled conditions		
Plant material information Type of plant material/product used in the experiment Plant identity (e.g. botanical name, variety) Conditions under which plant materials/products are managed Conditions of the plant commodity (e.g. degree of ripeness, presence of bark, etc.)		
Pest information Identity (species- strains biotypes if applicable-) Conditions under which the pests are cultured, reared or grown Method of infestation Level of infestation Stage of the pest that is most resistant to the treatment Was the most resistant stage used in the experiment? Potential development of resistance to the option Experiment(s) description and		(refer to research data if relevant)

analysis	
Variables used to measure efficacy	(e.g., mortality rate, count)
Factors influencing efficacy which were taken into account in the experiment	(e.g., wood humidity)
Factors influencing efficacy which were not taken into account in the experiment	(e.g., wood humidity)
Description of facilities and equipment	
Description of treatment	(e.g., temperature/duration, chemicals, concentration)
Monitoring of critical parameters	(e.g., number and placement of temperature sensors)
Description of experimental design	(e.g., randomization, blocks, number of replicates)
Presentation of the data	
Description of the statistical analysis	(e.g., anova, regression, test)
Conclusions of the experiment	
Other relevant information	

1.2. Experimental assessment of the option efficacy to reduce pest infestation in plant material/product under operational conditions

Source (indicate the reference of the supporting documents and data and their confidentiality status if applicable):

Item	***Description based on the submitted document(s)***	***Comments***
Experimental assessment of the option efficacy to reduce pest infestation in plant material/product under operational conditions		
Plant material information Type of plant material/product used in the experiment Plant identity (e.g. botanical name, variety) Conditions under which plant materials/products are managed Conditions of the plant commodity (e.g. degree of ripeness, presence of bark, etc.)		
Pest information Identity (species- strains biotypes if applicable-) Conditions under which the pests are cultured, reared or grown Method of infestation Level of infestation Stage of the pest that is most resistant to the treatment Was the most resistant stage used in the experiment?		(refer to research data if relevant)

Potential development of resistance to the option		
Experiment(s) description and analysis Variables used to measure efficacy Factors influencing efficacy which were taken into account in the experiment Factors influencing efficacy which were **not** taken into account in the experiment Description of facilities and equipment Description of treatment Monitoring of critical parameters Description of experimental design Presentation of the data Description of the statistical analysis Conclusions of the experiment Other relevant information	 (e.g., mortality rate, count) (e.g., wood humidity) (e.g., wood humidity) (e.g., temperature/duration, chemicals, concentration) (e.g., number and placement of temperature sensors) (e.g., randomization, blocks, number of replicates) (e.g., anova, regression, test)	

1.3. Analysis of the applicability of the risk reduction option

Source (indicate the reference of the supporting documents and data and their confidentiality status if applicable):

Item	***Description based on the submitted document(s)***	***Comments***
Analysis of the applicability of the risk reduction option		
Plan of implementation Place of implementation Characteristics of the treated material Description of the required facilities and equipments The degree to which the proposed option complements other phytosanitary measures Consideration of potential indirect effects	 (e.g., maximum size of the lot) (e.g. potential for the treatment to be used as part of a systems approach for one pest or to complement treatments for other pests) (e.g. impacts on the environment, impacts on non-target organisms, human and animal health)	
Monitoring of the plan Parameters that will be monitored Critical thresholds considered for these parameters Equipments used for the	 (e.g., wood temperature, presence of pest) (e.g., minimum temperature value) (e.g., temperature probes, detection	

monitoring Other relevant information	techniques)

1.4. Assessment of option effectiveness to reduce risk of pest entry from infested area to pest free area

Source (indicate the reference of the supporting documents and data and their confidentiality status if applicable):

Item	*Description based on the submitted document(s)*	*Comments*
Assessment of option effectiveness to reduce risk of pest entry from infested area to pest free area		
Consignments Origin Type of commodities Surveillance method Level of infestation of plant material/product Quantity of commodities Means of transportation	 (e.g., survey) (e.g., boats, planes, trains, tourisms)	
Detection method of the pest in the plant material/product Place(s) of implementation Sampling technique Type of detection method Accuracy	 (e.g., truck, harbor) (e.g., size, unit, number of samples) (e.g., visual inspection, laboratory test) (e.g., sensitivity, specificity)	
Point(s) of entry	(e.g., city)	
Variable used to describe probability of pest entry Conclusion of the assessment Other relevant information	(e.g., entry rate, probability, score)	

ABBREVIATIONS

APHIS	Animal and Plant Health Inspection Service
EFSA	European Food Safety Authority
EU	European Union
EPPO	European and Mediterranean Plant Protection Organisation
MS	Member State(s)
PLH	Plant Health
RH	relative humidity
USDA	United States Department of Agriculture
Xac	*Xanthomonas axonopodis* pv. *citri*